MICRO-ORGANISMS AND DISEASE

AN

INTRODUCTION INTO THE STUDY OF
SPECIFIC MICRO-ORGANISMS

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PREFACE TO THE THIRD EDITION.

A LITTLE over a year has passed since this book appeared, and a third edition has become necessary. In this I have made a number of alterations and additions. Some of the methods of preparing nutritive-media have been simplified, while others have been described more in detail. The methods of staining have been revised, and a number of facts bearing on septic and specific organisms, gained during the past year or two, have been added. I trust that this revision will add to the value of the work.

E. KLEIN.

November, 1885.

PREFACE.

THE following book, with few additions, is a reprint of a series of articles that have appeared in the *Practitioner* (1884). Not pretending to be in any sense an exhaustive treatise it will, I hope, nevertheless serve to illustrate most of the important points bearing on investigations into the life history of micro-organisms connected with infectious diseases.

I must not omit to mention that most of the investigations recorded here were carried out for the Medical Department of the Local Government Board during the past ten years.

E. KLEIN.

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CHAPTER I.

MICROSCOPIC EXAMINATION.

For the examination of micro-organisms good high powers are essential, at the least a power magnifying 300 to 400 linear diameters. Zeiss' D or E and Zeiss' or Powell and Lealand's oil immersion 1-12th or 1-16th inch will be found sufficient for all purposes. In the case of tissues stained with aniline dyes a good substage-condenser such as Abbé's or Powell and Lealand's, is invaluable. I use Zeiss' stand with Abbé's condenser, open diaphragm, and plane mirror. As Koch[1] pointed out, and what is now universally acted upon, stained specimens mounted in Canada-balsam solution or Dammar varnish, when examined on an Abbé's condenser, show the micro-organisms with extreme clearness and sharpness.

The examination of the morphological characters of an organism is carried out on fresh unstained, as well as on fresh stained, microscopic specimens. Although the latter method is, for reasons hereafter to be mentioned, by far the most perfect and reliable one, it is nevertheless important to

[1] *Die Aetiologie d. Wundinfectionskrankheiten*, p. 34, Leipzig, 1879 Translated as *Traumatic Infective Diseases* (New Syd. Soc.), London, 1880.

ascertain as far as possible the appearances, chemical reactions, and general morphology of perfectly fresh specimens. Blood, juices, tissues, and fluids in which the micro-organisms have been growing, are subjected directly, without any previous preparation, to microscopic examination. With artificial nourishing media in which micro-organisms have been growing, the examination of fresh specimens is of great importance, for the reason that the organisms can be easily identified and their size and general morphological characters be more correctly ascertained than after drying, hardening, and staining. Besides, the chemical reactions can be satisfactorily studied in fresh specimens only. All one has to do is to draw up with a capillary pipette or to take up with the point of a needle a drop or particle of the material, to place it on an object-glass, and to cover it up with a thin cover-glass. Where one has to deal with liquids, such as artificial nourishing fluids, blood serum, tissue-juices, secretions, transudations and exudations, no addition is required. In the case of more solid material, such as solid artificial nourishing material, bits of tissue, &c., the addition of a drop of neutral previously well-boiled saline solution (of 0·6 to 0·75 per cent.) is advantageous although not absolutely necessary, since by pressing down the cover-glass a layer of the material sufficiently thin for examination can be obtained. In some instances a bit of tissue can be teased out into fine particles by means of two clean needles. Where it is a question of micro-organisms sufficiently conspicuous by their shape, size, and general appearance, their identification in the fresh condition is not difficult; this is the case with bacilli, actinomyces, and mycelia, but in the case of micrococci, especially when isolated or in couples, and lying in blood, juices, or tissues, their recognition is often extremely difficult. When in large clumps, such as

larger or smaller masses of zoogloea, or when in the shape of chains, the identification is not difficult; but in the more isolated state they are not easily recognised, owing, as a rule, to the presence of granules or particles of various kinds, from which morphologically their distinction is well-nigh impossible. In such cases there are certain rules of thumb, if I may say so, which assist, although they do not absolutely insure, the diagnosis. These are the micro-chemical reactions. The addition of liquor potassæ leaves micro-organisms quite unaltered, whereas fatty and most albuminous granules alter or altogether disappear by it. Acetic acid from 5 to 10 per cent. strong does not affect micro-organisms, but albuminous and other granules become in most instances altered. These two re-agents, I think, are as reliable as any others; if they fail, then others like alcohol, chloroform, sulphuric ether, &c., are not of any greater help, but the latter re-agents may be used, for instance, when it is a question between fat-granules and micrococci, or crystals and bacilli.

Micro-organisms have a great affinity for certain dyes, especially aniline dyes, and therefore these are used with great success to demonstrate their presence, and to differentiate in many instances morphological details which in the unstained condition are not discernible. The staining is effected on fresh unaltered organisms, or after they have been dried. In the first instance the process is carried out thus:—A microscopic specimen is made, and to it is added afterwards drop after drop of the dye, passing it through the specimen in the usual way of applying fluids to a microscopic specimen, *i.e.* by adding with a capillary pipette the dye at one margin of the cover-glass and sucking it up with a strip of filter-paper applied to the opposite margin of the cover-glass. When the staining has taken

place the excess of the dye is washed away with salt solution, water or alcohol, or both, as the case may be (see below). Unless the organisms are embedded in continuous masses of solids, this method gives good results. In the latter case, say if they are embedded in a microscopic lump of tissue, or in a particular spot of a fine section of a fresh tissue, it is necessary, after having placed the lump or section on an object-glass, to drop the dye on to this previous to putting on the cover-glass. After some minutes the dye is allowed to run off by inclining the object-glass, and then the washing is proceeded with till all the excess of the dye is removed; the mounting is then done by placing a drop of water or salt solution on the specimen and covering it with a cover-glass. In the case of sections through fresh and hardened tissues containing micro-organisms, the method of staining and of permanently mounting them as a whole is more complicated, and will be detailed presently.

When one has to deal with coherent masses of micro-organisms, present either in natural media (*i.e.* animal tissue) or artificial cultivations, such as zoogloea and pellicles of *micrococcus* or *bacterium*, these can be bodily transferred to a watch-glass, stained, washed, and mounted without much difficulty, either for immediate or permanent use. The permanent specimens are made in this way:—Place the section or pellicle in a watch-glass containing the dye, leave it there till deeply tinted, take out with a needle or the like, wash in water, then in alcohol, leave here for five minutes or more till most of the excess of the colouring-matter is removed, then lift it on to an object-glass, spread well out, place on it a drop of clove-oil, and after a minute or two drain off the clove-oil, add a drop of Canada-balsam solution (in chloroform or benzol), and cover with a cover-glass. In

some special instances, such as the bacilli of leprosy and tuberculosis, double staining is required. With other organisms, such as the bacilli of glanders or tuberculosis, the washing is carried out, not with water but with acid (acetic acid and nitric acid respectively). All the details will be stated when dealing with these special organisms.

The method extensively and successfully used for the demonstration and preservation of microscopic specimens of micro-organisms in fluids, as blood, pus, and juices, is that of Weigert and Koch, which consists in spreading out on a glass slide or cover-glass a very thin film—the thinner the better—of the fluid (artificial or natural culture medium), blood, pus, or juice, and drying it rapidly by holding it for ten to twenty seconds over the flame of a spirit-lamp or gas-burner. The most successful preparations are obtained when the heating is carried on for such a time that the film, having become opaque at first, rapidly turns transparent. Several drops of the aniline dye to be used are then poured over the specimen, and after remaining on it from five to thirty minutes or more are poured off.

The cover-glass specimen is then well rinsed with distilled water, dried over the flame, and mounted in Canada-balsam solution or Dammar varnish—of course always bearing in mind on which surface of the cover-glass the film has been spread. If the film has been well dried in the first instance washing in water is quite sufficient, but if the drying has been insufficient a good deal of diffuse staining of the ground substance has taken place, and then of course the cover-glass specimen must be also washed in alcohol sufficiently long to remove this undesirable staining, then washed in water, dried and mounted. In some instances washing with alcohol removes also the dye from the bacteria, but as a rule it is better to first over-stain the cover-glass specimen, then wash

well in alcohol so as to remove the dye from all except the bacteria, but do not wash with alcohol too long, then rinse in distilled water, dry and mount.

The most useful dyes in the examination of animal tissues for bacteria are those aniline dyes that are soluble in water; these are preferable to those soluble in alcohol only. They have all great affinity for cell nuclei (Hermann) and belong to the group of neutral or basic aniline colours. Methyl-blue, methyl-violet, vesuvin, Bismarck-brown, magenta, fuchsin, gentian-violet, Spiller's purple, rosaniline, Humboldt's red (purple), are the dyes most commendable.

For staining of cover-glass specimens, as well as for sections made of fresh tissues, the above dyes can be advantageously used in the following manner: 2 to 5 grammes of the solid dye are rubbed up in a mortar with 10 ccm. of absolute alcohol; add then gradually, while mixing, warm distilled water, to bring up the total to 100 ccm.; filter and keep in stoppered bottle. For use, filter a little of the dye into a watch-glass. For staining sections of tissues that have been hardened, the above dyes prepared with Weigert's aniline oil are preferable; they are prepared thus: (*a*) Make a saturated watery solution of pure aniline (aniline oil) by mixing in a bottle one part of aniline oil with three parts of distilled water; shake well every half hour for four to six hours, decant the water as the oil settles to the bottom. The decanted fluid is the saturated watery solution of aniline. Of this take 100 ccm. Add to this (*b*) a saturated alcoholic solution of fuchsin, magenta, gentian-violet, Humboldt's red, methyl-blue or methyl-violet, 11 ccm.; mix well, filter into stoppered bottle. The sections are left in this dye for from a few minutes to several hours (Humboldt's red requires only a few seconds). Different bacteria require different periods to stain. As a rule warming the dye

facilitates the staining of the bacteria; occasionally, also, the addition of a few drops of liquor potassæ. All sections, after having been sufficiently stained, are transferred to and washed in water, then methylated spirit, then in absolute alcohol, then clarified in clove-oil, and finally mounted in Canada-balsam (dissolved in chloroform, or better still in benzol) or in Dammar varnish.

In order to bring out by the dye more conspicuously the bacteria present in fluids or tissues various methods are used, all of which are based on the principle that the bacteria have an affinity to the dye which is greater than that of the tissue-elements. Hence after staining, the tissue-elements may be decolourised without abstracting the colour from the acteria. Cover-glass specimens or sections, after having een well stained with a dye, are subjected to various lecolourising re-agents, whereby the tissue-elements become leprived of the dye, but the bacteria retain it. Although in ome instances this is not easy of achievement, since by uch decolourising processes also the bacteria are liable to ose the stain, it nevertheless is possible in the majority f instances. In many cases prolonged washing in alcohol bsolutus and in clove-oil is sufficient to abstract the dye om the tissue-elements, but in some special cases, owing to eculiar chemical properties possessed by certain bacteria, e decolourising process requires special methods. Of these e following are the most useful:—

1. In some instances the specimens (cover-glass specimens and particularly sections) are stained in one dye, then washed in alcohol till quite pale, then transferred to a contrast dye. As contrast dyes are to be regarded blue and red, or red and brown, or blue and brown, or violet and brown. In some cases only the bacteria retain the first dye, the tissue-elements become stained by the second dye.

A similar result is often obtained by mixing the two dyes, and then using them like a single dye; hereby occasionally the bacteria are found to take one colour, while the tissue-elements take the contrast dye.

2. One of the most useful methods for staining bacteria in sections of hardened tissues is Gram's method. Sections are kept for ten minutes in absolute alcohol, are then placed in any of the above mixtures of aniline oil and dye (fuchsin, magenta, Humboldt's red or gentian-violet, methyl-blue or methyl-violet), and kept here for from two to five minutes or more; they are then washed in alcohol for from one to three minutes, and are then transferred into the following solution: one part of iodine, two parts of iodide of potash, 300 parts of distilled water; they are kept here till their colour completely changes (as a rule into dark purple), they are then transferred into alcohol till all colour has apparently gone. If successful, such sections when examined under the microscope, show only the bacteria stained, while the tissue-elements are quite colourless. To bring out these latter more strikingly the sections are stained in a contrast dye, vesuvin or Bismarck-brown, if red, violet, or blue has been used as the first dye.

3. Ehrlich's method, used specially for demonstrating tubercle-bacilli and leprosy-bacilli.—The specimens, after having been well stained (from a few minutes to several hours) with fuchsin or magenta aniline oil dye (see above), are transferred into a 30 per cent. mixture of nitric acid; according to Friedländer a mixture of three parts of nitric acid in 100 parts of alcohol is equally good. A 10 per cent. watery solution of nitric acid is quite strong enough. All bacteria except the tubercle-bacilli and leprosy-bacilli lose the dye by this treatment. The preparations are then stained for contrast in vesuvin or Bismarck-brown.

For other methods to stain tubercle-bacilli see the chapter on the latter.

4. Koch's method.—According to this the sections, after having been stained, are transferred to a saturated solution of carbonate of potash to which previously an equal volume of water has been added. The preparations remain here for from five to ten minutes, are then washed in water, alcohol, clove-oil, and finally mounted in Canada-balsam solution or Dammar varnish.

5. Lustgarten's[1] method, used for the demonstration of the syphilis-bacilli.—The sections are stained for from twelve to twenty-four hours at ordinary temperature, and then for an additional two hours at 40° C. in aniline oil gentian-violet; they are then washed for a few minutes in absolute alcohol, and then transferred to a 1·5 per cent. solution of permanganate of potash for ten seconds, then for the same period into a watery solution of pure sulphurous acid; wash in distilled water, repeat the above process of placing the sections first into the permanganate of potash solution, then into the sulphurous acid water till they become apparently quite colourless. Only the syphilis-bacilli, tubercle-bacilli and leprosy-bacilli, are able to retain the dye; other bacteria lose it by being subjected to the permanganate.

De Giacomi[2] has improved this method of decolourising by oxidation. Cover-glass specimens made of syphilis material are stained with warm fuchsin for a few minutes, are then washed in water to which a few drops of solution of iron perchloride have been added, then placed into concentrated solution of iron perchloride till the preparations have lost all colour; they are then stained for contrast in vesuvin or Bismarck-brown.

[1] Lustgarten, *Med. Jahrbücher der K.K. Ges. d. Aerzte*, Vienna, 1885.
[2] De Giacomi, *Schweizer Correspondezblatt*, xv. 12.

A. Gottstein[1] places sections of syphilis material for twenty-four hours in fuchsin or aniline oil gentian-violet; wash with distilled water, then place them for a few seconds into a pure or dilute solution of liquor ferri, then wash in alcohol, clarify in clove-oil, mount in Canada-balsam.

It may not be unnecessary to point out, that if sections are kept for many hours in the staining fluid, there may be found in them micro-organisms (particularly bacilli) which have been accidentally introduced into them by the solutions of aniline dye. Many of these, particularly when used alkaline, contain organisms, and if the sections are kept in them for many hours, notably in warm weather, bacteria will be found to have not only invaded the tissue but to have multiplied therein.

In examining fresh or hardened tissues for micro-organisms it is necessary to make thin sections, which can be easily done with the aid of any of the microtomes in common use, amongst which Williams's microtome, and especially Dr. Roy's ether-spray freezing microtome, are no doubt the best and easiest to manipulate. As regards hardened material, it is necessary to remember that the hardening must be carried out properly, small bits—about a half to one cubic inch—of tissue being placed in alcohol, or better, in Müller's fluid, and kept there; in the first instance, for two to five days; in the second, for from one to three weeks or more. Then small bits are cut out, of which it is desired to make sections. Those hardened in spirit must be soaked well in water to enable the material to freeze, then superficially dried with blotting-paper, and then used for cutting sections with Roy's microtome. Those hardened in Müller's fluid are at once superficially dried with blotting-paper and cut. When making sections with Williams's freezing microtome it is necessary to soak the material first in gum mucilage and then

[1] A. Gottstein, *Fortschritte d. Medizin*, Berlin, 1885, No. 16, p. 545.

end a fine hole. When the temperature of the water rises, the mercurial column rises, and at a certain temperature rises above the lower open end of the small inner glass tube just mentioned. If this point is reached, then the burner at *C* receives only the amount of gas that passes through the fine lateral hole of that inner glass tube. If the temperature of the water falls, the mercury falls, and the lower end of the inner glass tube becomes again free, and now the burner at *C* receives a much greater supply of gas. If so, the temperature of the water again rises, the mercury rises, obstructs the lower end of the inner glass tube, the supply of gas is reduced to what can pass through the fine lateral hole, and consequently the temperature again falls, and so on. To adjust the regulator it is necessary when the thermometer indicates the required degree of temperature to push the outer large glass tube, and with it the inner tube, of the regulator so far down that the top of the mercurial column just obstructs the free end of the inner glass. The temperature then regulates itself for the reasons stated previously. These regulators are sufficient for all practical purposes when it is not a question of small differences in temperature, since they are tolerably constant within one or two centigrades. The trouble one experiences in the working of these and other similar regulators arises from the inconstancy of the main gas supply, this, as is well known, varying within wide limits. The stopcock, *E*, obviates this to a limited extent; when this is put at an angle of 45° only a limited amount of gas passes from the main supply tube to the regulator, and therefore the variations in pressure of the gas are not felt to their full extent. A Sugg's regulator interposed between *E* and the main supply tap is very useful.

B. Thermometer to indicate the temperature in the chamber.

C. Gas burner.

D. Chamber of incubator.

E. Stopcock to regulate, when required, the supply of gas.

F. Main supply.—The upper, lower, right and left walls of the incubator are made of a double layer of tin; between the two is water. The front and back of the chamber are closed by a movable glass plate.

An excellent incubator for constant temperature is made by the Cambridge Scientific Instrument Company. It has a double gas supply: one small permanent flame, and a second one subject to the regulator.

varying between 20° and 38° C., are necessary in order to study more accurately the life-history of the septic as well as the pathogenic organisms. Moreover, large numbers of them become available in a short time, and their relation to disease can be tested more conveniently. For if it should be found that, having carried on outside the animal body successive cultivations of a particular organism, the re-introduction of this cultivated organism into the animal body is again productive of the same disorder as before, then the conclusion becomes inevitable that this organism is intimately related to the causation of the disease. It must be conceded that after several successive cultivations in fluids any hypothetical substance supposed to be the *materies morbi*, and introduced at first from the blood or tissues, being in a very diluted condition in the first cultivation, would after several

cultivations be practically lost. But if this last cultivation should be found to act in the same manner pathogenically, *i.e.* if every droplet of it, charged with the new brood of the organism, nevertheless possesses full pathogenic power, then it is logical to say that this pathogenic property rests with the organism. For this and other reasons it is of essential importance to be able to carry on successive cultivations of one and the same organism without any accidental contamination or admixture, *i.e.* it is necessary to carry on *pure cultivations.*

ARTIFICIAL CULTIVATION MEDIA.

A.—FLUIDS.

As fluid nourishing material the following are used with preference :—

1. *Broth made from Meat—pork, beef, rabbit, chicken.*—The connective tissue and fat are first cut out from the fresh meat —in the case of rabbit or chicken the whole animal without head or viscera is used—and then placed in water and boiled. Generally for each pound half an hour's good boiling is allowed. With regard to the quantity of water, each pound of meat ought to yield ultimately at least one pint of broth. When boiled, the broth is allowed to stand, the fat is skimmed off, and the broth well neutralised, or even made faintly alkaline by adding liquor potassæ, or, better still, carbonate of sodium.

The fresher the meat the less acid (sarcolactic acid) is in the broth before neutralisation. The broth is then filtered through a filter, previously overheated (see below), into flasks previously sterilised (see below). As a rule beef broth is

in all other cases, sterilised by overheating) into flasks, and treated in the same manner as the broth.

An excellent fluid is beef broth with 1 per cent. of peptone, the mixture is then made faintly alkaline, boiled and filtered into sterile flasks, then well boiled to serve as stock, or to be at once decanted into test-tubes.

3. *Buchner's Fluid.*—10 parts of Liebig's extract, and 8 parts of peptone, in 1,000 parts of water.

4. *Hydrocele Fluid* (Koch).—A new or well sterilised (by over-heating) trocar and cannula are used for the tapping; to the cannula is fixed an india-rubber tube that has been soaking in strong carbolic acid solution for forty-eight hours. The distal end of the tube is introduced carefully and rapidly into the neck of a sterilised flask plugged with sterile cotton-wool, and the fluid thus allowed to flow into the flask to about two-thirds of its volume. This fluid is then decanted into sterile test-tubes (plugged with sterile cotton-wool), each tube receiving about 5 to 8 ccm. The tubes are then exposed in the incubator to a temperature of from 55° to 60° C. for three to five hours on five or six consecutive days.

5. *Blood Serum* (Koch).—A glass cannula and india-rubber tubing are soaked for forty-eight hours in strong carbolic acid; the cannula is tied into the carotid artery of a healthy horse, and the arterial blood, after opening the clip at the proximal end of the artery, is allowed to flow into sterile flasks, or cylinders with stoppers. After letting the blood stand for 12 to 24 hours in a refrigerator or in an ice box, the serum is taken off by means of large sterile glass pipettes and introduced into sterile test-tubes, each receiving about 5 to 8 ccm. The test-tubes, plugged with sterile cotton-wool, are then exposed in the incubator to a temperature of 58° to 62° C. in the same manner and for the same time as the hydrocele fluid was.

6. *Urine* is neutralised and sterilised by boiling for 20 to 30 minutes like broth.

7. *Milk* (ordinary) is sterilised by gentle and careful boiling for 20 to 30 minutes.

Of less common use are:

8. *Pasteur's Fluid.*—In 100 parts of distilled water are dissolved 10 parts of pure cane-sugar, 1 part of ammonium tartrate, and the ash of 1 part of yeast.

9. *Cohn's Fluid.*—100 ccm. of distilled water, 1 gramme of ammonium tartrate, no sugar, and instead of the ash of yeast are substituted (A. Mayer) 0·5 gramme of potassium phosphate, 0·5 gramme of crystallised magnesium sulphate, 0·05 gramme of (tribasic) calcium phosphate. These two fluids are sterilised in the same manner as the broth and peptone solutions. Pathogenic organisms do not thrive in either of these two fluids.

B.—Solids.

The solid media have the great advantage over the fluids that in the former artificial cultures can be carried out more easily; as, owing to the resistance the solid basis offers to the growth of the organisms, they remain more limited to the spot or spots on which they are sown, and therefore can be watched more easily; besides, an accidental contamination, *i.e.* a growth appearing at a spot at which no sowing was made, can be recognised at once. These advantages are perhaps of the greatest use when it is intended to grow the organisms on a surface exposed to the influence of air—of course protected from contamination with other organisms.

These advantages of solid media have been very minutely

pointed out by Koch in his researches on pathogenic bacteria.[1]

As solid media are used:

1. *Slices of Boiled Potato or Boiled White of Egg or Paste* (Fokker, Schröter, Cohn, Wernich).—A boiled potato or a boiled unshelled egg is cut in half with sterile scalpel, and the cut surface is inoculated. Immediately after, it is placed on a clean glass plate and covered with a bell-glass, the edges of the latter being fixed on the former by vaseline or grease, the chamber is kept moist by a piece of wet blotting paper being placed inside the bell-glass. The progress of the growth of a particular organism or of different organisms sown out at a particular spot or line on the surface of these substances can be easily watched with the unaided eye.

2. *Gelatine* (Brefeld, Grawitz, Koch).—This is used advantageously as a mixture with broth, peptone, beef-extract, blood serum, or hydrocele fluid. Koch, who introduced this mixture, used it for the cultivation of bacteria on solids, to be exposed to the air; the proportion of gelatine in the mixture was 2 to 3 per cent. But this mixture, although solid at ordinary temperature, does not keep solid in the incubator, not even at 20° C. I have found that at least 7·5 per cent. of gelatine must be contained in the mixture to keep it solid at 20° to 25° C. Above this temperature not even 11 per cent. gelatine will keep solid.

Nutrient Gelatine, most useful for the growth of all kinds of bacteria, is prepared in this way:

One pound of lean beef is cut up, to it is added one pint of water, and is kept boiling in the digestor or any other vessel for from half to three-quarters of an hour. After having been strained through fine calico it is filtered through paper into a beaker; bring up by adding water to 600 ccm.;

[1] *Mittheilungen d. k. Gesundheitsamtes*, i. 1881.

add to this 60 grams of the finest gold label gelatine cut up in small pieces, 6 grams of peptone and 6 grams of common salt. Dissolve on waterbath, but do not let the water boil; neutralise with carbonate of soda or, better, liquor potassae till faintly alkaline; boil for half an hour, filter by hot filter, (see Fig. 6) into a sterile flask plugged with sterile cotton-wool, and bring it up to boiling point, at which it is kept for a few minutes. This can be kept as stock gelatine, or can be decanted at once into sterile test-tubes plugged with sterile cotton-wool. Keeps solid up to about 25° C.

Prepared in this manner the nutrient gelatine passes easily and comparatively rapidly through filter paper on hot-filter.

The same 10 per cent. nutrient gelatine can be of course obtained if broth is already made, *e.g.* broth in a stock flask, by adding the above-named quantities of gelatine peptone and salt to 600 ccm. of the broth; further process is as above.

3. If it is necessary to expose the cultivation to higher temperatures than 25° C., the nutrient gelatine cannot be used as a solid medium. Solid blood serum or solid hydrocele fluid (Makins) or solid Agar-Agar mixture (Koch) must then be employed.

The first, *i.e.* the serum of blood, and the second, *i.e.* the hydrocele fluid, can be made solid by heating the above sterile serum or hydrocele fluid in tubes (see page 20) *gradually* up to 68° or 70° C. When this temperature is reached the material soon turns solid, losing slightly its limpidity, but is sufficiently transparent for all practical purposes. By heating it rapidly, or heating it above 72°, it becomes solid, granular, and opaque. Of course, once thus made solid it cannot be liquefied again, and therefore must be already contained in the vessels (test-tubes and small flasks) in which the growth of organisms is to be carried on. Or blood serum

and hydrocele fluid can be rendered solid by exposing the sterilised material (see above), in sterile plugged test-tubes, to a moderate heat—*e.g.* in the incubator at 32° to 38° C.—for several weeks. Through evaporation the material is rendered solid. Thus treated it retains its limpidity in a perfect manner.

AGAR-AGAR, or Japan isinglass, can be obtained[1] in the shape of thin transparent lamellæ, or more usually as masses of transparent narrow bands. For cultivation purposes the following mixture is prepared :

1. Place in a beaker Agar-Agar 10 grams, common salt 10 grams, distilled water 900 ccm. Dissolve over the flame and boil for twenty minutes, strain, and filter through hot-filter into a sterile flask plugged with sterile cotton-wool.

It ought to be mentioned, that in all cases where any material (broth, peptone sugar, nutrient gelatine, or Agar-Agar solution) is filtered into a sterile flask the cotton-wool plug is taken off, and the glass tube of the filter having been inserted into the neck of the flask, the cotton-wool is so replaced that it well guards the mouth of the flask.

2. Place into a clean beaker 100 ccm. of distilled water, add 10 grams of peptone, and two small tins of Brand's meat extract ; dissolve over flame, add liquor potassae till faintly alkaline, boil, filter, and add the filtrate to the above Agar-Agar solution. For the two tins of Brand's, 10 grams of Liebig's meat extract can be substituted. Thus a mixture is obtained which contains, besides meat extract, 1 per cent. of Agar-Agar, 1 per cent. of peptone and 1 per cent. of salt. This is then boiled for half an hour and can be kept as stock material or can be at once decanted into sterile test-tubes.

[1] Messrs. Christy and Co., of 155 Fenchurch Street, have succeeded in obtaining for me large quantities of this material from Paris. I understand from my friend Dr. R. Maddox that this substance is in reality what the French call *Gelose*.

It keeps solid up to a temperature of 50° C., *i.e.* a temperature higher than is ever necessary for the growth of bacteria. It becomes liquid at higher temperatures, and in case of necessity can be again subjected to boiling. Before considering it as perfectly sterile it ought to be kept like all other materials for from several days to several weeks in the incubator at 32° to 38° C. If quite limpid after this time it may safely be considered as sterile.

Amongst all the solid media, I have found this mixture of Agar-Agar and peptone meat extract to be excellent in many respects. It is beautifully limpid and solid, and an excellent nourishing material. Agar-Agar alone without the admixture of peptone is not satisfactory as a culture medium.

The materials which I now prefer for the cultivation of pathogenic and septic bacteria are: 1. Broth (beef) and peptone; 2. Solid nutritive gelatine; 3. Solid Agar-Agar peptone meat extract; 4. Fluid and solid serum of blood; all faintly alkaline.

CHAPTER III.

VESSELS AND INSTRUMENTS USED IN CULTIVATIONS.

ALL *vessels* (flasks, test-tubes, beakers, filters, calico) to be used are first thoroughly sterilised by overheating. In the case of flasks and test-tubes, this can be done by exposing them thoroughly in *all parts* to the open flame of a large Fletcher's burner; *while thoroughly heated* the mouth is plugged with a good long plug (1 to 2 inches) of sterile cotton-wool, this being pushed in by means of overheated forceps. The plug in all cases must not be loose, but also not too firm—an error in the latter direction being of course preferable to one in the former. The cotton-wool plug may, if long enough, be single; or, if short ones are used, double. Or the flasks and test-tubes are placed in an air-chamber (see Fig. 4) heated by a large Fletcher's burner for several hours, up to between 130° and 150° C. In the case of small flasks and test-tubes this process is of course much more convenient, since a large number can be heated simultaneously. Beakers and glass filters to be used merely for a temporary operation are placed over a wire net on a tripod and heated by the flame of a Bunsen's burner. In the case of test-tubes which are to receive cultivation-fluids, I generally expose them, after having been cleaned and dried, in the air-

chamber for several hours (three to six) to a temperature of from 130° to 150° C.: while hot they are taken out *seriatim*, plugged with the sterile cotton-wool, and replaced in the air-chamber, and heated again for several hours. All this, and other operations to be described below, may appear to some rather tedious and unnecessarily complicated, but it cannot be too strongly insisted on that in these matters one cannot

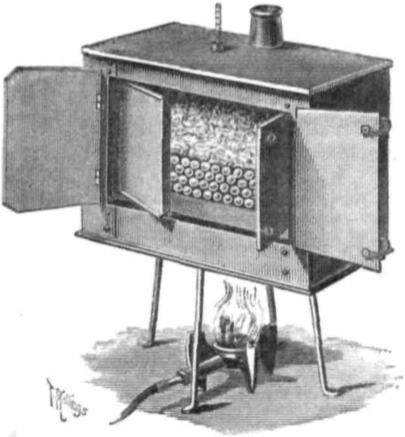

FIG. 4.—HOT-AIR CHAMBER FOR STERILISING TEST-TUBES AND COTTON-WOOL.

An iron chamber with double wall, the inner chamber having separate folding doors. In the inner chamber are placed the test-tubes, glasses, &c., and the cotton-wool, the latter in a loose condition. Both sets of doors are closed, and the apparatus heated by a large Fletcher's burner. A thermometer passing from the inner chamber through the upper wall indicates the temperature of the chamber.

be too scrupulous. A slight relaxation may, and occasionally is, followed by disastrous consequences in the shape of accidental contamination, and consequent loss of material prepared at the cost of much labour and time. Long experience in these matters has taught me that, although in some instances less scrupulous care has not been followed

by bad results, still I have had also many unpleasant failures owing to slight laxity in these matters.

Several weeks' work may be annihilated by a single omission. Sometimes one is perhaps in a slight hurry, and does not think the want of an additional heating of the test-tube or cotton-wool or an additional boiling of the fluid will be followed by any bad consequences. But, alas, nature does not take into account our convenience, and failure is our reward. If in any kind of experiments "overdoing" is an error in the right direction, it is in these very experiments in the cultivation of micro-organisms.

The *cotton-wool* used for plugging flasks and test-tubes is prepared by pulling up loosely a quantity of good cotton-wool and exposing it in a loose state in the air-chamber to a temperature of 130° to 150° C. *for several hours for several successive days.* The cotton-wool ought to be just brown, *i.e.* just singed. Too much charring makes it brittle, and it is then difficult to make of it a satisfactory plug. The plug used should not be too firm and not too loose: in the former case it is not easy to lift it up quickly, and in the latter it does not close sufficiently well. Cotton-wool that has been kept, say only for a day or two in the air-chamber for three or four hours is not absolutely sterile; nor is cotton-wool that has been kept in a compressed state in the air-chamber for any number of days. The central portions remain under these conditions quite white and are not sterile. No cotton-wool that is not just brown, *i.e.* just singed, is safe from risk of impurity. No cotton-wool steeped in absolute alcohol, strong carbolic acid, or any other disinfecting fluid, for ever so many days or weeks, can be absolutely relied on.

As stated above, a plug of sterile cotton-wool tolerably firm, of about one to two inches, or two plugs of about one

inch each, are used for the plugging of the flasks and test-tubes. An assertion such as that made by Dr. Williams at the British Association (Biological Section, September 1883), that cotton-wool plugs are not reliable, because they do not protect the fluids in the vessels plugged with them from accidental air-contamination, is to be accepted only as applying to very loose plugs and to cotton-wool not properly sterilised. To good firm plugs of sterile cotton-wool it evidently cannot apply, since all the results of all workers in this field (Pasteur, Sanderson, Cohn, Koch, Klebs, Buchner, and many others) are against it.

Instruments, such as the points of needles, and forceps, used in the processes of cultivation, lifting up cotton-wool plugs, making cotton-wool plugs, inoculations, &c., must be heated in the open flame of a Bunsen burner, if they are to be absolutely relied on for cleanliness. Scissors and knives used for cutting tissues which are intended for inoculation, ought to be likewise scrupulously clean. One ought to keep a special set of instruments, the blades of which are capable of being heated in the open flame without being spoilt.

Syringes used for cutaneous, subcutaneous, or other inoculations, ought to be capable of being overheated. The ordinary Pravaz syringe of vulcanite not being capable of undergoing this process, Koch has devised a glass syringe similar to the Pravaz syringe. I do not use any syringe for inoculation, but prefer using each time a fresh *capillary glass pipette* made just before the inoculation. Into this

FIG. 5—A CAPILLARY PIPETTE, DRAWN OUT INTO FINE POINTS; LENGTH ABOUT 12 TO 14 INCHES.

pipette I draw the droplet to be used for inoculation, and having made a very small incision—about $\frac{1}{8}$ of an inch—through the skin, the pointed end of the pipette is pushed forward into the subcutaneous tissue for about half an inch or one inch and then the fluid is blown out into the tissue.

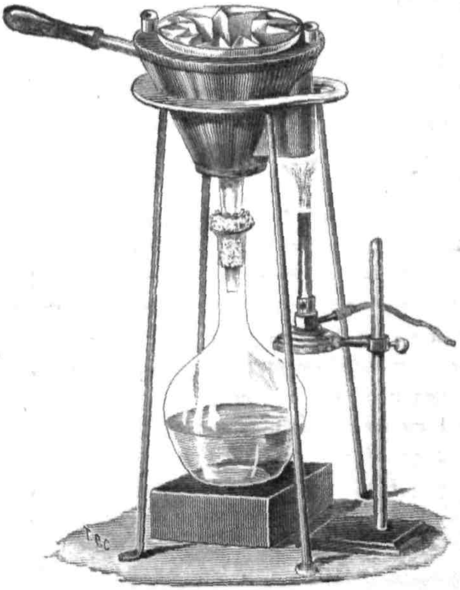

FIG. 6.—HOT-WATER FILTER FOR FILTERING NUTRITIVE GELATINE OR AGAR-AGAR MIXTURE.

In this way I am always absolutely safe from any contamination with a previously used virus, which might possibly adhere to one or other part of a syringe.

The fine point of capillary pipettes (Fig. 5), used for inoculation of animals, or for drawing out a drop of fluid of a

cultivation in a flask or test-tube, or for inoculating material contained in a test-tube or flask, are thus made: while one hand holds the bulb of the pipette, the other holds one end, and putting at some distance from this end the tube into an

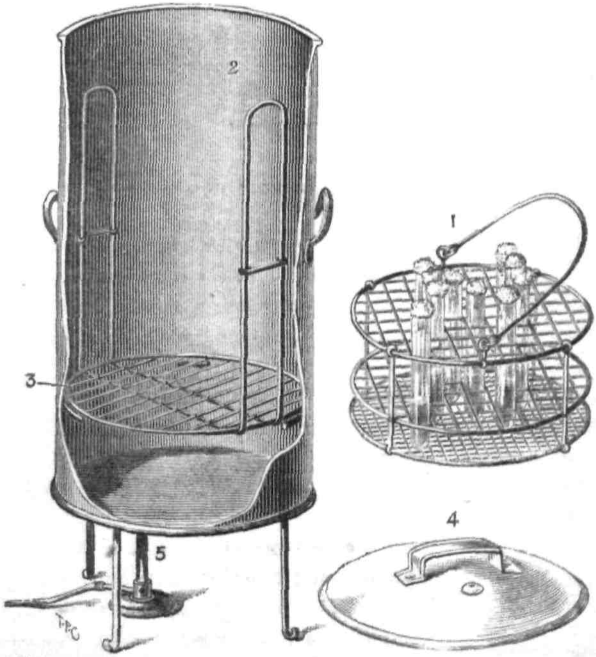

FIG. 7.—STEAMER FOR STERILISING CULTURE MATERIAL CONTAINED IN TEST-TUBES.

1. Wire-net to hold the test-tubes; 2. Tin vessel; 3. Wire diaphragm to hold 1; underneath it is water; 4. Lid; 5. Gas-burner.

ordinary flame and quickly drawing it out, a point of extreme fineness can be made. The same is done with the other end. Such a pipette can be considered as practically closed at both ends.

CHAPTER IV.

PREPARATION OF CULTURE-MEDIA FOR INOCULATION.

WE have on a former page described the methods to obtain sterile stock of nourishing media suitable for artificial cultivations. The solids, as serum gelatine, serum and hydrocele fluid, must, before solidification, be placed in test-tubes and small flasks, and then sterilised in the manner above described, to be made ready for establishing cultures, *i.e.* for inoculation. The Agar-Agar mixture however can, like broth, peptone mixture, beef extract solution, and gelatine mixtures, be kept as stock in large flasks. When thus sterile these latter can be decanted into a number of test-tubes or small flasks, in which the cultivation is to be carried out. Gelatine mixtures (gelatine and broth, gelatine and peptone, gelatine and beef-extract) and the Agar-Agar mixture, must of course be liquefied over a flame before being ready for decanting. The test-tubes most suitable are about six inches long, and should not be less than about one inch broad ; the flasks are about of the capacity of one to two ounces, and ought to have a neck of comparatively good width. The test-tubes receive the fluids for about one and a-half to two and a-half inches in depth, the flasks for about one-fourth to one-third of their bulk. All these test-tubes and flasks with

their cotton-wool plugs, before receiving the material, should be thoroughly sterilised by overheating. As I mentioned in the previous chapter, this ought to be well borne in mind, for starting with a sterile nourishing fluid—*i.e.* one that has been kept in the stock flask for several days to several weeks in the incubator at a temperature of from 32° to 38° C. and that has remained perfectly clear and limpid—and working with thoroughly sterilised test-tubes and cotton-wool plugs—very little care is required to obtain sterile material ready for inoculation. To start with a stock of nourishing material, however well sterilised, and to decant it into test-tubes with cotton-wool plugs not absolutely sterile must lead to failure. I have seen this happen over and over again, and all the material decanted became consequently contaminated and thereby useless for inoculations. The test tubes and flasks must be well cleaned, then dried, placed in the air-chamber, and kept there exposed for several hours to a temperature of from 130° to 150° C. on several successive days, or they may be thoroughly heated in all parts over the open flame of a gas-burner. The same applies to the cotton-wool, as mentioned in a former chapter. The test-tubes and flasks are plugged by means of clean forceps with the cotton-wool which is just brown, and then replaced in the air-chamber and again heated for several hours on two or three occasions up to a temperature of 130° to 150° C., or they may be well heated over the open flame of the burner. To decant sterile stock fluid into these test-tubes and flasks I proceed thus: A clean beaker with spout, covered with a clean glass plate, is placed on a wire net on a tripod over the flame of a Bunsen burner, and thoroughly heated for half an hour or so; then it is allowed to cool, and when cool, the plug of the stock flask is lifted with forceps, and some of the sterile fluid quickly poured from the flask into the beaker. The

plug is replaced in the neck of the stock flask and the beaker covered with the glass plate. Of course the quantity poured into the beaker should be large enough to supply the required number of test-tubes or small flasks. The stock flask containing still some fluid, having been opened for however short a time, has of course been exposed to air-contamination, and therefore must be treated accordingly, if the fluid left in it is to serve as sterile nourishing material on a future occasion. Consequently it is subjected to boiling for from fifteen to thirty minutes, then placed in the incubator, boiled again the next day and put back in the incubator, where it is left at a temperature of from 32° to 38° C. for several days. If after a week or so the fluid remains limpid, it is of course to be considered sterile.

Next, the fluid that has been poured into the beaker (covered with the glass plate) is poured as quickly as possible into the test-tubes, one after the other, by lifting with clean forceps the plug and pouring in the fluid to a depth of one and a half to two and a half inches, and the plug replaced.

During this procedure contamination with air-organisms, if there be any about, becomes inevitable. To lessen this chance as much as possible, it is necessary to lift the plug with clean forceps, to pour the fluid as rapidly as is practicable into the test-tube or flask, and to replace immediately the cotton-wool plug. Further, it is necessary to bear in mind, that the atmosphere is not at all times and everywhere equally contaminated (see Prof. Tyndall's observations). I generally avoid undertaking this process on windy days, and when I do it, I generally close windows and doors and keep the air in the room as still as possible. I do not do it in a room in which recently (say an hour or two previously) the floor, walls, or tables have been swept.

I have opened under these conditions the plugs of test-tubes containing sterile material, and kept them so for a time varying from one to ten seconds, and in some instances I have not seen more than from 10 to 20 per cent. contaminated, often less.

Now, having filled the required number of test-tubes and flasks with the desired quantity of fluid, I subject these *seriatim* to boiling. By means of an ordinary test-tube

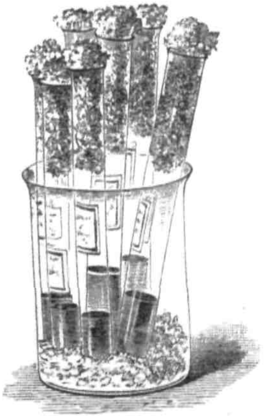

FIG. 8.—A BEAKER CONTAINING A NUMBER OF CULTURE-TUBES PLUGGED WITH COTTON-WOOL.

holder I hold them above a very small flame until the fluid boils, and keep it so boiling for from two to five minutes. During this process of boiling the cotton-wool is only slightly pulled up, and immediately before ceasing to boil the plug is again replaced, aud pushed down with a clean glass rod. Then the test-tube is placed (of course upright) in a beaker at the bottom of which a layer of cotton-wool—a sort of

cushion—has been placed. When finished, the test-tubes in the beaker are all transferred to the incubator and kept there for from twelve to twenty-four hours at a temperature of 32° to 38° C. Then the boiling is repeated once more. After this they are kept in the incubator for several days to several weeks. I generally keep them there from two to three weeks, and all those in which the fluid has remained limpid and clear are considered sterile and ready for use. As a rule, starting with sterile stock fluid, and using thoroughly sterile test-tubes and cotton-wool plugs, after once or twice boiling after decanting there ought to be no loss of tubes through accidental contamination with air-organisms (during decanting). Sometimes, however, I have had loss to the amount of 5 per cent. or more, but then there was always a hitch of some kind traceable. To decant under carbolic acid spray is not practicable and possesses many unpleasant drawbacks, besides, in some instances when I used it there was really a greater percentage of contaminated tubes than without it. I therefore do not use the spray.

A simple method is to subject the whole number of test-tubes into which the nutritive material had been decanted (broth, peptone broth, nutritive gelatine, Agar-Agar mixture,) to a steamer (see Fig. 7). The test-tubes are placed into the wire net (see figure), the top of the group of test-tubes is covered with tinfoil, so as to protect the plugs from becoming wet, and then the wire net is placed into the steamer—the water at the bottom of which has been previously heated to boiling,—the lid is put on and the steaming is kept up for from fifteen to twenty minutes. This is repeated on one or two successive days. I have not seen any tubes go bad, after they have thus been steamed on three successive days each time for twenty minutes. Placed in the incubator and kept at a temperature of 35° to 38° C., from some days to one

or two weeks, they remain free of any growth and are to be considered sterile.

Test-tubes containing solid nourishing material are generally kept sufficiently inclined during solidification of the material (see a former chapter) to allow the material to spread into a layer of large area; although useful, it is not essential.

When test-tubes with sterile fluid blood-serum are to be subjected to the process of solidification (see page 23), it is advisable to keep the tubes in a slanting position, so as to allow the serum to spread out into a layer which is sufficiently transparent even after solidification. For this purpose I place these test-tubes into a double-walled tin trough, about five inches broad, twenty inches long; between the two walls and also inside the trough is water of the temperature of 62° to 65° C. The test-tubes are placed side by side, so that they rest with the bottom part on the floor, with the top part on the opposite ledge of the trough. The difference of level is such, that it allows the serum to spread out in the test-tubes into a large area. By means of burners the temperature of the water is then gradually raised to 68° to 70° C.

In order to protect the solid materials, contained in test-tubes, from drying up, it is advantageous to keep them in large wide-mouthed bottles with well-fitting (by vaseline, grease, or otherwise) glass stoppers. This is particularly necessary in the case of test-tubes, containing Agar-Agar mixture or blood-serum, and which are to be kept in the incubator for many days or weeks at a temperature of 35° to 3 C.

CHAPTER V.

METHODS OF INOCULATION.

HAVING now in test-tubes and small flasks sterile material ready for inoculation, it is necessary to describe the mode of inoculating the same.

1. *Inoculations from Artificial Cultures.*—The first and simplest is the case where it is required to inoculate a new tube or flask with a definite organism that has been growing previously in a culture-tube; that is to say, where it is required to establish from an artificial cultivation a new and further artificial cultivation. Take a freshly drawn-out capillary pipette, with a fine point, as described in a former chapter; draw up with clean forceps slightly the top part of the cotton-wool plug of the old tube or flask, push carefully and gently one of the pointed ends of the capillary pipette—the other can be broken off blunt—through the remaining part of the cotton-wool plug, and push it downwards till it emerges into the culture-fluid, or, if this be solid material, till it reaches the spot or place where the organism is growing; allow a small droplet to ascend into the capillary pipette, which it readily does by capillarity; or if a larger quantity is required draw it up by gently sucking at the outer end of the capillary pipette. Then draw the capillary pipette

altogether out of the tube and cotton-wool plug, and push this latter down with the forceps into its former position. Immediately after this proceed to inoculate the new culture-tube by doing exactly the same as before, viz., draw up slightly with the forceps the top part of its cotton-wool plug, push through the remainder of this plug the pointed end of the capillary pipette, *i.e.* the one containing the droplet of the material to be sown, and push it into the material at the bottom of the test-tube or flask. A trace of the sowing material flows out by itself, or, if a large quantity is required, it is carefully blown from the pipette, but, of course, not so that the tube is emptied by the blowing. If the sowing is to be carried out on the surface of solid material, the seed is deposited on the surface; if in the depth, the end of the pipette is pushed down into the depth of the material and the seed there deposited. The pipette is then altogether withdrawn and the plug replaced as before. The new tube is then placed in a beaker on a cushion of cotton-wool, and exposed to the required temperature in the incubator.

If we have, however, a culture-fluid or any fluid that contains, as the microscopical examination proves, various species of organisms, which we wish to isolate, then the method of Klebs of "fractional cultivation," or the method of Lister and v. Nägeli of "dilution," or better still, Koch's method of "plate-cultivation," is resorted to.

The "fractional cultivation" consists in the attempt to isolate by successive cultivations the different organisms that have been growing previously in the same culture. If we take up by means of a capillary pipette a trace of the culture-fluid, and inoculate with traces of it in the manner above described a series of new culture-tubes containing various nourishing material, and expose these tubes in the incubator to a definite temperature, say 35° C., then the chances are

that in the first twenty-four or forty-eight hours not all the different species of organisms sown out will have increased equally in numbers in all tubes; most probably only one species in each tube, *i.e.* the one that grows best in this particular medium and at this particular temperature, will be found to have increased to an enormous extent, while the others have made little or no progress as yet. The nourishing fluid appears turbid, and filled chiefly with the one kind of organism. Now draw out with a fresh capillary pipette a minute droplet of this new culture and inoculate with a trace of it a new culture-tube. The chances are that you inoculate only one kind, that is, the one which is most abundant or perhaps is solely present. After twenty-four hours' incubation this new tube contains now probably only one kind of organism. To make it quite certain, inoculate from this a new culture-tube in the same manner, and now you probably have sown only a single species. In this manner by continued transferrence it is possible to obtain cultures of only one species of organisms. Many conditions, such as naked-eye appearances of a particular kind, coloration of the culture-medium, formation of a pellicle, the quantity of growth in a given time, soon indicate whether we have the desired single species; in some instances it is, however, extremely difficult to isolate after this method.

The method of "dilution" means diluting the culture-fluid containing the various species to a very large extent with some sterile indifferent fluid, such as well-boiled saline solution of 0·6 per cent., and then inoculating new tubes with a droplet of this greatly diluted material. For this purpose draw into a rather large pipette a tiny droplet of the old culture-fluid, then pass the pointed end of this pipette into a test-tube or flask (plugged) containing well-boiled saline solution, and draw up a quantity of this solution so as to

greatly dilute (1000-fold or more) the droplet of culture-fluid, and with this inoculate then a series of new culture-tubes containing different nourishing material, using always only a trace for inoculation. In this way it is probable that, owing to the great dilution, the trace of a droplet of this mixture used for the new inoculation contains only one species. Using a series of new culture-tubes and inoculating them thus, after twenty-four hours of incubation it will be found that some tubes have not received any seed, others only one species. If it be required to dilute the original fluid greatly, say if it teems with different organisms, then a droplet of this is placed into a large flask containing the well-boiled saline solution, so that a dilution of 1 in 1,000,000 or more can be effected.

The two methods, *i.e.* that of fractional culture and of dilution, may be successfully combined in this way: from the first or second new culture, established after the method of fractional cultivation, in which after twenty-four or thirty-six hours one species greatly predominates, draw out with a large capillary pipette a droplet, and dilute this to a great extent with the saline solution, as described above, and now inoculate with a trace of this mixture a new culture-tube. Or, if after twenty-four hours' incubation the microscope reveals in this further culture more than one species, continue the process of dilution and inoculation for a further generation. Thus it is possible to obtain cultures of only one species, although the original fluid contained several species of organisms.

One of the best methods for isolation is that of the plate-cultivation introduced by Koch in connection with the isolation of the choleraic comma bacilli. A test-tube containing sterile nutritive gelatine as above prepared is liquefied by gentle heat, best by being kept in water of about

40° C., then the plug is lifted and the gelatine inoculated with a mere trace of the bacterial mixture, either by means of the point of the capillary pipette or of the overheated and cooled point of a platinum wire ; the plug is replaced and the gelatine shaken so as to distribute uniformly the bacteria that had been introduced. A shallow glass dish with flat bottom and ground edge, and covered with a similar but slightly larger dish, has previously been sterilised in the oven and then allowed to cool ; the liquefied nutrient gelatine inoculated with the trace of the bacterial material is then poured out into the lower dish so as to form a thin layer at its bottom ; the lifting off of the dish-cover, the pouring in of the gelatine, and the replacing of the cover, ought to occupy only a moment.

This apparatus is then placed on a glass plate, to which, by means of greased edge, a bell-glass can be fixed, on the interior of which is a piece of wet blotting-paper. In this way a closed moist chamber is established. The whole is then put into an incubator, the temperature of which does not reach above 21° or 22° C. (or the temperature of the room in the warm months), in order to insure the gelatine setting and remaining so. If a trace of material containing various species of bacteria is thus distributed into several cc. of gelatine, each species will start a separate colony after a few days' growth, and the individual colonies, if different, will be apparent by different characters, according to shape, colour, size of the colonies, and according to whether they liquefy the gelatine or not during their growth. In order to insure success, it is necessary to infect the original gelatine in the test-tubes with only a trace of the bacterial mixture ; if too many bacteria are introduced, their colonies sprouting up are too numerous and soon become confluent. But if the experiment is successful, from the individual and separate

colonies, it is then easy by re-inoculation of gelatine tubes, or other nutritive material, to start pure subcultures of the different species. It must be borne in mind that not all bacteria can be isolated by this method, for some species of pathogenic organisms require for this growth a higher temperature than the one at which the nutrient gelatine

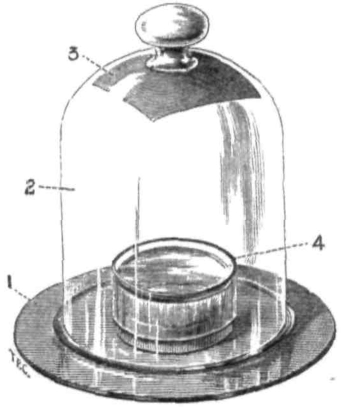

FIG. 9.—PLATE-CULTIVATION.

1. Glass plate.
2. Bell-glass.
3. Wet filter paper.
4. Glass dish containing the plate-cultivation on a thin layer of nutritive gelatine. This glass dish is covered by a second glass dish.

remains solid, while others refuse altogether to grow in gelatine, or grow only two slow. In the latter case no success can be looked for, since those bacteria which grow much faster crowd out the others that require a long time to come up.

In such cases, particularly when one has to deal with

bacteria that do not grow in gelatine at the temperature at which this latter remains solid, the same method of plate-cultivation can be used, but substituting the gelatine by the Agar-Agar mixture above mentioned, care must be taken not to proceed with the inoculation of the Agar-Agar mixture before the temperature has fallen to about 50° C. All other manipulations remain the same.

2. *Inoculations with Blood, Juices, and Tissues.*—To establish a cultivation from blood of a dead animal, cut open the thorax by removing the sternum with clean scissors, cut open the pericardial sac, pierce with the pointed end of a fresh capillary pipette the wall of the right ventricle or right auricle, and allow a drop or two of blood to ascend into the pipette, or if a larger quantity is required suck it up. Withdraw the pipette and inoculate new culture-tubes as above. Or, if blood of a large vein is required, separate the vessel with clean instruments, and make a small incision with clean scissors and push the pointed end of the capillary pipette well forward. If juice of a lymphatic gland, or spleen, or other parenchymatous organ be required, pierce the organ after having washed its surface with strong solution of perchloride of mercury (Koch), with the pointed end of a capillary pipette, then push it into the part required for a little distance, and squeezing the organ press a drop or two of the juice into it. The same procedure is adopted when the pus of an abscess is required, the wall of which can be pierced with the pointed end of the capillary pipette. If not, a slight incision is made and the pipette introduced through this into the abscess. If blood of a living animal is required, expose a vessel with clean instruments, make a small incision with clean scissors, push through this incision the pointed end of the capillary pipette well forward, and allow the blood to rise into the capillary tube. If blood of a living human being is required,

clean well with soap and water and then with strong carbolic acid or perchloride of mercury solution the tip of a finger, make a venous congestion in the last phalanx by compressing it with a corner of a handkerchief, prick the volar skin of the phalanx with a clean (overheated and cooled) needle, and plunging the pointed end of the pipette into the drop of blood, allow a droplet to ascend into the capillary tube of the pipette.

If solid tissues or parts of tissues are required, *e.g.* the base of an ulcer, a tubercle of the liver, spleen, or lung, it is possible to squeeze into the capillary tube of a pipette, after pushing its pointed end into the part, a small droplet of juice of the part required; but if this be not practicable, *i.e.* if a solid particle be required, then follow Koch's method. This is as follows: Cut with clean scissors or scalpel into the part, dig out rapidly with the point of a needle or platinum wire previously overheated in the flame of a burner a small particle, and quickly introduce this into the culture-tube to the place required, *e.g.* surface or depth of a solid or fluid nourishing material. Of course in this case the cotton-wool must be altogether lifted, and therefore contamination with organisms is possible. But inoculating several tubes at once and performing the operation quickly, one always succeeds in getting some of the tubes without any air-contamination. I have made numerous inoculations with solid particles (tubercles) in this manner, and like Koch have seen only a small percentage of tubes going bad through contamination with air-organisms.

The same plan, *i.e.* of using the clean point of a needle or platinum wire for taking up the material to be used for inoculation, is resorted to if one has to deal with the culture in solid nourishing material, on or in which the organisms are growing that we want to transplant either for inoculation

of a new tube or of an animal. A useful method, which does not require the lifting out of the plug at all, and which can easily be employed in the last case, is this; deposit from the pointed end of a capillary pipette a droplet of some sterile fluid (broth or thoroughly-boiled saline solution) on the spot of the solid medium on which the organisms are growing, then scratch this spot with the end of the capillary pipette in order to get the organisms off from the solid basis and mixed with the drop of fluid deposited there, then let this drop again ascend into the end of the capillary pipette, and withdraw this altogether. All this can be done without lifting out the cotton-wool plug of the test-tube or flask in which the growth is proceeding.

If one has to use a particle of tissue the surrounding portions of which are probably contaminated by putrefactive organisms, *e.g.* a tubercle in the lung or a tubercle in the spleen, it is well to follow Koch, and to disinfect the surrounding parts by just washing them with a dilute solution of corrosive sublimate, and then to remove these parts with clean scissors so as to obtain the central particle which one wishes to use for inoculation: of course one must not steep the whole organ in sublimate solution, since this would naturally destroy all organisms.

All these methods can be easily modified according to the requirements of the special cases, and it is not necessary here to give more than what has already been described in the preceding.[1]

In order to observe in a microscopic specimen the gradual changes in the growth of a micro-organism, there are several methods employed. In all of them it is of

[1] Compare also Koch, *Untersuchungen über pathogene Bacterien*, in *Berichte aus dem k. Gesundheitsamte*, Berlin, 1881; and *Die Aetiologie d. Tuberculose*, *Berlin. klin. Wochenschrift*, No. 15, 1882.

course necessary to keep the specimen heated up to the desired temperature.

The simplest method consists in sowing the organisms on a suitable nourishing material in a small glass cell, fit to be placed on the stage of a microscope and to be there observed even with high powers, similar to those cells which Koch has used in his studies on bacillus anthracis. Such a glass cell consists of a glass slide, in its centre a concave pit, not too large, and capable of being quite closed up by an ordinary cover-glass, the edges of which fasten by means of clean paraffin or olive oil. Place with a clean needle a speck of spleen pulp of an animal dead of anthrax into a drop of nourishing material, fluid or solid, on the centre of a clean cover-glass, the edges of which have been prepared as just mentioned, and fasten this on the above slide so that the specimen faces the concave pit: expose this so prepared specimen to a constant temperature, either by placing it in the incubator and examining it with the microscope from hour to hour, or on the warm stage (Stricker, Ranvier) used in histological work for directly observing the influence of temperature on the various cells and tissues; or, place it simply on the stage of the microscope and expose the whole (*i.e.* microscope and all) in a suitable warm chamber (after Klebs), but so that the chamber allows light to pass by means of a small window to the mirror of the microscope, while the eyepiece is so arranged as to project through a hole in the upper wall of the chamber. The plan which I generally follow is with slight modifications that of Koch.

A glass cell (Fig. 10) is made by cementing a glass ring, $\frac{3}{4}$-$\frac{7}{8}$ inch in diameter and about $\frac{1}{8}$-$\frac{1}{12}$ inch high, on to an ordinary glass slip. The chamber of this cell is well cleaned with absolute alcohol. A thin cover-glass, square or round,

about one inch in breadth, is well heated by holding it for a few seconds over the flame of a gas-burner or spirit-lamp. On the upper edge of the above glass ring is placed with a camels' hair brush a thin layer of clean olive oil; a droplet of water is deposited on the bottom of the cell in order to keep this afterwards well supplied with moisture; a drop of the sterile nourishing material (broth, aqueous humour, hydrocele fluid, blood-serum, liquefied gelatine mixture, liquefied Agar-Agar mixture, &c.,) is then deposited by means

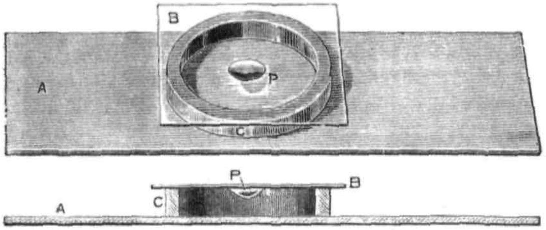

FIG. 10.—A GLASS CELL, FOR OBSERVING UNDER THE MICROSCOPE THE PROGRESS OF GROWTH OF MICRO-ORGANISMS.

The upper figure shows the cell in perspective; the lower figure in profile or cross section.

A. Glass slide.
B. Cover-glass.
C. Glass ring forming the wall of the chamber.
P. Drop of nourishing material in which the micro-organisms grow.

of a capillary pipette on to the centre of the cover-glass; then the point of a capillary pipette or needle containing the material it is desired to sow is rapidly plunged into the drop of the nourishing material (or if this is solidified is deposited in lines or points on the drop of nourishing material), the cover-glass inverted and placed on to the glass ring: the layer of olive oil keeps the edges of the cover-glass air-tight on the glass ring. This cell is then placed into the incubator

and exposed there to the desired temperature. Microscopic examination is carried out from time to time to watch the progress made. This can be done with high powers, since the growth is taking place on the lower surface of the cover-glass.

Although contamination with air-organisms is not excluded, still it is possible by making several specimens at the same time and operating rapidly, to obtain pure cultures. This glass cell can be also watched on a warm stage, or in a Klebs' warm chamber.

M. Nachet of Paris has designed a glass cell, in which the drop of nourishing material is deposited on to the bottom of the cell, the glass slip being here replaced by a very thin glass; but then there is a peculiar arrangement in the microscope, by which the lower surface of the glass cell, *i.e.* the one nearest to the growth, is directly subjected to microscopic observation.

After what has been said above about inoculation of solid and fluid nourishing media with solid matter, it is not necessary to dwell specially on the method of inoculation with earth or similar substances.

3. *Examination of Water for Micro-organisms.*—Most water contains bacteria of some kind, as has been shown by direct experiment by Burdon-Sanderson.[1] If any sample of water is to be examined for micro-organisms, particularly bacterial forms, it is allowed to stand for a few hours, till most of the particulate matter is settled, and then with a capillary pipette a little of the fluid and sediment is drawn out and used for (*a*) microscopic specimens to be examined fresh; (*b*) microscopic specimens prepared after the Weigert-Koch method, *i.e.* by spreading out on a cover-glass a thin layer, drying it, staining it with suitable aniline dyes, *e.g.*

[1] *Reports of the Medical Officer of the Privy Council*, 1870.

Spiller's purple, gentian violet, methyl blue, or magenta, washing with water, then spirit, then distilled water, then drying, and finally mounting it in Canada-balsam solution. (*c*) Test-tubes containing sterile nourishing material (broth, Agar-Agar mixture, gelatine mixture, Cohn's or Pasteur's fluid) are inoculated in the manner described previously, *i.e.* by piercing the cotton-wool plug with the pointed end of the capillary pipette. These test-tubes are then exposed in the incubator, and after one or two days or more, a sample is withdrawn with a capillary pipette, and used for microscopic examination. As a rule, after a day or two of incubation we can already distinguish with the unaided eye whether there are any organisms present, the nourishing fluid either being uniformly turbid—this is generally the case—or there being a growth at the bottom of the fluid. But of course the microscopic examination only shows what kind of organisms are present. New cultivations are made from this one, if any are required. (*d*) A good plan of recognising easily that there are present various kinds of organisms in such cultures is one similar to that recommended by Professor Angus Smith.[1] Sterile gelatine broth or gelatine only, contained in sterile test-tubes plugged with sterile cotton-wool, is liquefied, but of course not heated to more than about 35° to 40° C. then inoculated with the water (to be tested), by means of the capillary pipette; after inoculation the gelatine is mixed by shaking the test-tube slightly. In this way the organisms present in the water are distributed in the gelatine. Then the gelatine is allowed to set and is kept in this solid state. The organisms being distributed in the gelatine, after some days' growth are noticeable as clusters which gradually increase in extent and are distributed in various parts of the

[1] *Sanitary Record*, p. 344, 1883.

medium. The various species, owing to difference of growth, form clusters differing in aspect, size, and arrangement.

A good plan of ascertaining the relative number of certain bacteria present in given samples of water is that by means of plate-cultivations. A definite small quantity of the water is added to a definite quantity of liquefied sterile nutrient gelatine contained in a sterile test-tube; shake well and pour out on a glass plate or glass dish to be kept under bell-glass in a moist chamber, *i.e.* after the manner of an ordinary plate cultivation. After being kept at a temperature of about 21° to 22° C. notice the number of colonies that have sprung up, the number of different colonies (size, shape, colour, and whether liquefying the gelatine or not), and from these an index is obtained of the approximate number of such bacteria that have been originally present in a given quantity of the water. But it must be borne in mind that the number of colonies is no absolute index of the number of bacteria originally present in the water, for the following reasons: (*a*) not every colony that makes its appearance on the plate cultivation—even granted it is due to the growth of a single species, but which is not always the case—owes its origin to one single individual, since for instance micrococci bacteria and bacilli may occur in the original water as zoogloea and chains, and these cannot by any amount of shaking be broken up into single elements; (*b*) not all bacteria introduced into the gelatine come up as colonies, since not all of them are capable of growing in the gelatine, and not all can thrive at the temperature at which the gelatine remains solid; (*c*) the liquefaction of the gelatine by some of the colonies and not by others does not indicate different species, since this depends sometimes on the nature of the nutrient gelatine, and to the fact whether the growth takes place in the depth or on the surface; (*d*) accidental contamination with organisms

of the air during the preparation of the plate cultivation cannot be prevented, and if the air happens to contain a good many organisms, the total number of colonies appearing in the plate cultivation exceeds the number of bacteria present in the water tested.

Professor Warden of Calcutta (*Chemical News*, Nos. 1340 to 1344) and Dr. Percy Frankland (*Proceedings of the Royal Society*, No. 238) have made valuable experiments on the examination of bacteria present in water by means of plate cultivations.

4. *Examination of Air.*—The simplest plan to test for the presence of organisms in the air is to draw out the cotton-wool plug of several test-tubes or flasks containing the sterile nourishing material, or, if this be boiled, potatoe, paste, or gelatine (see p. 20), to expose their surface, and to leave it thus for variable periods, from a few seconds to several minutes. Then replace everything and expose the material to incubation, or keep it only at the ordinary temperature of the room. Another method is to collect the particles present in the air on glasses moistened with pure glycerine (Maddox), and then to make microscopic specimens or inoculate tubes with this glycerine.

A method which is very useful is the one recommended by Cohn and Miflet.[1] The principle of it is, that by means of an aspirator, an air-pump of any kind—*e.g.* a Sprengel pump, or simply the fall of water—air of a particular locality is drawn into one, two, or more Wolff's bottles (each with the ordinary two bent glass tubes), connected with one another by short pieces of indiarubber tubing, and containing the sterile material in which the organisms are required to grow. All bottles and tubes are of course sterile; the plugging of the tubes after the air has passed is done with sterile cotton-wool.

[1] *Zeitschr. f. Biol. d. Pfl.* iii. 1, p. 119.

Any given quantity of air for any length of time can be passed through a series of such bottles, the one that receives the air first being of course most contaminated.

The bottles are after the experiment placed in the incubator, if required, the outer end of their tubes being plugged with cotton-wool.

Miquel[1] has carefully described many ingenious methods for the study of air-organisms.

[1] *Les Organismes vivants de l'Atmosphère*, Paris, 1883.

CHAPTER VI.

MORPHOLOGY OF BACTERIA.

BACTERIA are minute organisms not containing chlorophyll, and multiplying by fission—hence the term *schizomycetes* (v. Nägeli). They are composed of a kind of protoplasm, the mycoprotein of Nencki, and are invested with a membrane, which is composed chiefly of cellulose and a certain amount of mycoprotein (Nencki).

Their contents are transparent and clear, but sometimes contain minute bright granules of sulphur (Beggiatoa). Owing to the cellulose membrane they resist the action of acids and alkalies. Many species of bacteria—micrococcus, bacterium, spirillum—are able by rapid multiplication to form colonies; the individuals are then embedded in a hyaline gelatinous matrix produced by them, this is also mycoprotein, Some species are possessed of one or two straight or slightly spiral cilia or flagella, and thereby they are capable of locomotion, darting through, or spinning round, in the fluid in which they are suspended. Such is the case with some kinds of bacteria, bacilli, and spirilla.

Bacteria grow best when left undisturbed; movement of the vessel in which they grow is not advantageous. Light and electricity do not appear to have a decided influence on some bacteria, since they grow well in the light. According to Cohn and Mendelssohn,[1] strong electric currents have a noxious influence on the growth of micrococci.

[1] Cohn's *Beitr. z. Biol. d. Pfl.* Bd. iii. 1.

Engelmann[1] describes a bacterium photometricum, the motility of which directly depends on light; it ceases in the dark. Duclaux found that exposure to direct sunlight injures the life and growth of some bacteria, both septic and pathogenic.

Some bacteria require free access of oxygen, and are called aërobic (Pasteur); others grow without free oxygen, and are anaërobic (Pasteur). All require for their growth certain nourishing material containing carbon and nitrogen. Water is an essential element for them, and a certain temperature is in many instances a stimulant of their growth. Most pathogenic bacteria require for their propagation a temperature varying in the different cases between 18° and 40° C. The bacteria obtain their nitrogen from organic compounds; some are capable of obtaining it from compounds as simple as ammonium tartrate; others, especially pathogenic organisms, require much more complex combinations, such as occur in the animal body. Carbon they obtain likewise from organic compounds, such as carbohydrates, amongst which sugar is the chief, and vegetable acids combined as salts are also to be mentioned. It is essential for all that certain inorganic salts, phosphates, potassium and sodium salts, should be present, since their own substance contains a large percentage of it—4 to 6 per cent.

While all are capable of disintegrating organic combinations containing nitrogen, they in their turn help to produce certain chemical products, which in some cases are definite for a definite species (see below). Such is the case with the various bacteria connected with the fermentations producing lactic acid, butyric acid, and acids belonging to the aromatic series. On many bacteria connected with putrefaction, and also on some pathogenic organisms, these chemical products

[1] *Unters. aus. d. physiol. Labor*, Utrecht, 1882.

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[1] *Unters. aus. d. physiol. Labor*, Utrecht, 1882.

have a deleterious effect. Small quantities impede their growth, and sufficiently large quantities kill them altogether.

Most bacteria are killed by heat below the temperature of boiling water, many of them when exposed for several hours to a temperature above 50° to 60° C. Exceptions are the spores of bacilli, which in some instances (spores of hay bacillus, Cohn) require exposure to the heat of boiling point for as much as half an hour. By raising the boiling point above 100°, it does not require more than a few minutes to kill them (Sanderson).

Drying destroys most bacteria, except the spores of bacilli. Freezing destroys likewise most bacteria, except the spores of bacilli, which survive exposure to as low a temperature as − 15° C., even when exposed for an hour or more. No spores survive exposure to a temperature of 120° C.

Amongst those substances which inhibit the growth of, or altogether destroy the bacteria, are carbolic acid, salicylic acid, thymol, &c.; corrosive sublimate is one of the most powerful, since even solutions as weak as 1 : 15,000 inhibit the growth of some bacteria after exposure for thirty minutes.

The best classification of bacteria is that given by Cohn,[1] and this we shall adopt: (1) spherobacteria or micrococci; (2) bacteria or microbacteria; (3) bacilli or desmobacteria; (4) spirilla, (5) spirochætæ. There are also various kinds which approach one or the other of these, *e.g.* ascococcus, sarcina, leptothrix (Beggiotoa), cladothrix, streptothrix, &c. (see below).

I shall not attempt to give an exhaustive description of the morphological characters of all micro-organisms, but shall limit myself to those forms which are related in some way or other to diseases.

[1] *Beitr. z. Biol. d. Pfl.* Bd. i.

CHAPTER VII.

MICROCOCCUS (Hallier, Cohn).

By the specific term micrococcus is understood a minute spherical or slightly oval organism (spherobacterium, Cohn), that like other bacteria divides by fission (schizomycetes), and that does not possess any special organ, cilium or flagellum, by using which it would be capable of moving freely about. Micrococci, like other granules when suspended in a fluid medium, show (Brownian) molecular movement. Micrococci propagate always by simple division, never by any other means, *e.g.* gemmation and spores. All assertions to the contrary are based on incorrect observations. All micrococci possess a delicate membrane of cellulose, and owing to this resist the action of alkalies and acids. The contents are homogeneous and highly refractive while active, pale when inactive. They consist like those of other bacteria of mycoprotein (Nencki). The size of micrococci varies within considerable limits, say 0·0005 to 0·002 millimetres, or even a little more. Micrococci vary greatly as regards both size and mode of growth. All multiply by slightly elongating and then dividing by a transverse constriction into two: a dumb-bell; each of these again divides into two, either transversely or in the same direction as before. The

new elements of successive divisions may remain connected, and thus form a chain (or mycothrix, Itzigsohn and Hallier; torulaform string, Cohn), or they separate into single organisms or dumb-bells. In some species there is a pre-eminent tendency to form chiefly dumb-bells, in others to form shorter or longer chains generally more or less curved, streptococcus (Billroth).

Such exquisite chains one meets with sometimes in serum of blood exposed to the air for some days, and in pleural and

FIG. 11.—MICROCOCCI OF PUTRID HUMAN SPUTUM.

1. Single micrococci and dumb-bells.
2. Short chains.
3. A long chain.
4. A zooglœa.

This and all subsequent figures are drawn under a magnifying power of about 700 diameters except stated otherwise.

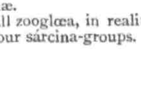

FIG. 12.—FROM THE SAME PUTRID SPUTUM AS IN PREVIOUS FIGURE. THE MICROCOCCI ARE LARGER.

1. Dumb-bells.
2. Sarcinæ.
3. A small zooglœa, in reality consisting of four sárcina-groups.

peritoneal exudations of animals dead for a few days. I have seen in an artificial culture made by my friend Mr. A. Lingard from a blister in a rabbit's ear the most exquisite convolutions of threads of micrococci. (See Fig. 13.)

A dumb-bell is also called a diplococcus (Billroth). Between the individuals of a dumb-bell there is always noticeable a short pale intervening bridge.

Some species are specially characterised by dividing into a dumb-bell; if each of the elements divides again transversely into a dumb-bell; a group of four (tetrade or sarcina-form) is thereby produced. Some species are occasionally

FIG. 13.—PART OF A CONVOLUTION OF CHAINS OF MICROCOCCI; FROM AN ARTIFICIAL CULTIVATION STARTED WITH THE SERUM OF A BLISTER OF A RABBIT'S EAR.

met with, particularly in products of air-contamination, in which the four individuals are closely pressed against one another, and then each assumes more or less the shape of a cube, a true sarcina (see below). But each of these cubes

FIG. 14.—GIANT MICROCOCCI, FROM SAME PUTRID SPUTUM AS IN PREVIOUS FIGURES.

1. Dumb-bells.
2. Division of dumb-bells into sarcina.
3. Incomplete division into sarcina.

FIG. 15.—SARCINA-MICROCOCCUS, FROM AN ARTIFICIAL CULTIVATION.

1. The elements of each sarcina-group of four appears single.
2. The elements incompletely divided into secondary groups.
3. Each element of the previous groups has divided into four small micrococci.

divides into four small micrococci arranged as a small sarcina, so that a sarcina-within-sarcina-form results.

In many instances the individual members resulting from division remain closely adherent without any definite arrange-

ment, and thus form smaller or larger continuous masses, *zooglœa* or colonies, in which the individuals appear embedded in a hyaline gelatinous matrix; the amount of this varies in the different species; in some there is little of the matrix actually visible, the micrococci being in close juxtaposition, in others it is easily recognised, the interstices between the individuals being measurable.

In some of the pigmented species (see below) the interstitial matrix contains the pigment. Zooglœa masses always present themselves as uniformly granular, the granules or micrococci being of the same size.

True micrococci never elongate to form rods, although in certain rod-like bacteria the individual elements sometimes assume the shape of spherical elements (see below).

Some species of micrococci form after some days a pellicle on the surface of fluid nourishing material, although there is also an abundance of these micrococci in the depth of the nourishing material. This pellicle is composed of zooglœa, and after some time bits of it, or the whole, sink to the bottom of the fluid medium. Micrococci that thus form pellicles are pre-eminently aërobic (Pasteur), *i.e.* require a great deal of free oxygen, which they receive from the air to which they are exposed on the surface of the nourishing material. Other species do not require free oxygen (anaërobic, Pasteur), and therefore grow well in the depth and do not form a superficial pellicle. There is a marked distinction in this respect between different species. The micrococci occurring in connection with disease are anaërobic.

When cultivated in suitable fluids, they produce after a day or two general turbidity; growing in solid nutritive gelatine some produce liquefaction of the gelatine, others do not.

Micrococci may be divided, according to their chemical

and physiological function, into: (*a*) septic, (*b*) zymogenic,[1] (*c*) chromogenic, and (*d*) pathogenic micrococci.

(*a*) The *septic micrococci* are micrococci that occur with other septic bacteria, wherever there is decomposition of organic matter in solids or in fluids. There exists a large number of species of such micrococci, differing from one another in size and mode of growth. They are widely distributed in the air, and contamination by air is often followed by the appearance of micrococci. They also occur in the body of man and animals wherever there is dead tissue, in which they grow well and copiously. Of this kind are the micrococci found in ordinary pus (Ogston), in the normal oral cavity (on the filiform papillæ of the tongue and on the mucous membrane), in the bronchial secretion in ordinary catarrhal exudations (nasal cavity, bronchi, &c.), on the free surface of intestinal and other ulcerations, in the cavity of the small and large intestine, and in the epidermis of the normal skin (Bizzozero).

(*b*) *Zymogenic micrococci* are micrococci associated with definite chemical processes. (α) Micrococcus ureæ, causing the ammoniacal fermentation of urine (aërobic, Pasteur), occurs singly, as dumb-bells or chains, and as zooglœa. (β) The micrococcus of the mucoid wine fermentation produces (Pasteur) a peculiar mucoid change in wine and beer, and occurs chiefly in chains. (γ) The micrococcus causing phosphorescence in putrid meat and fish (Pflüger) forms chiefly zooglœa (aërobic).

(*c*) *Chromogenic micrococci* (Schröter, Cohn).—These micrococci are characterised by their power of forming

[1] I adopt this term from Flügge: *Fermente und Mikroparasiten*, Leipzig, 1883.

pigment of various colours. They grow well at ordinary temperatures, and occur chiefly as zooglœa; they differ from one another by forming different pigments. The thicker the layer the more marked is the pigment. This is either soluble in water or it is insoluble, in the latter case it remains limited to the cells and their interstitial substance. The cells are spherical (Micrococcus prodigiosus, chlorinus, fulvus) or slightly elliptical (M. luteus, auriantiacus, cyaneus, violaceus). They are all aërobic and produce this pigment only when there is free access of air. They grow best on boiled potato, bread, paste, and boiled-egg albumen. They can be transplanted, and always produce the same pigment. When growing and kept in the depth of a solid nourishing material, *i.e.* removed from the free surface, they grow as

FIG. 16.—OVAL MICROCOCCI WHICH POSSESS A BLUE COLOUR, MICROCOCCUS CYANEUS, SINGLY AND IN DUMB-BELLS.

colourless micrococci. They abound in the air—in some localities and at certain seasons more than at others. (*α*) Micrococcus prodigiosus is blood-red, the colour is lodged not in the micrococci but in the interstitial substance, and is insoluble in water, soluble in alcohol; it occurs chiefly as zooglœa, in the shape of smaller or larger droplets. The cells are the smallest of all pigment-micrococci. (*β*) Micrococcus luteus is yellowish, and the pigment is insoluble in water. It occurs also in fluid nourishing material, forming a pellicle. I have met with it in the air, and have sown it in fluid pork-broth, where it grew very abundantly at a temperature of 32° to 38° C. It was found as single cells or dumb-bells, and formed a thick pellicle on the surface,

which after some time sank down into the fluid, the pellicle retaining a pale yellow colour. (γ) Micrococcus auriantiacus grows on boiled-egg albumen, chiefly as zooglœa. The pigment is soluble in water. (δ) Micrococcus cyaneus, violaceus, chlorinus, and fulvus, produce blue, violet, green, and brown pigment respectively. The first two grow well as zooglœa of elliptical cells on boiled potatoes, the third on boiled-egg albumen, and the last is met with on horses' dung.

Clathrocystis roseo-persicina (Cohn), peach-coloured bacterium, Bact. rubescens (Lankester), is an organism of about 0·0025 mm. in diameter, spherical or oval and of a bright red colour. The cells differ from micrococcus prodigiosus, not only in their greater size and their intrinsic colour, but also in this—that having formed zooglœa-masses there are gradually developed cavities or cysts therein, which are filled with water, while the coloured cells occupy the periphery. The cysts ultimately break up. Together with this organism occur other pink-coloured organisms described by Cohn as *monades*.

Monas vinosa, spherical cells about 0·002—0·003 mm. in diameter.

Monas Okenii, cylindrical cells, 0·008—0·005 mm. long, 0·005 mm. broad, flagellate.

Rhabdomonas rosea, spindle-shaped, 0·004 mm. broad, 0·02—0·03 mm. long, flagellate.

Monas Warmingii, spindle-shaped, 0·008 mm. broad, 0·015—0·020 mm. long, flagellate.

Ascococcus.—Billroth first described certain peculiar spherical, oval, or knobbed masses of minute micrococci, which he found in putrid meat infusion. Each of the masses is enveloped in a resistant firm hyaline capsule of about 0·010

to 0·015 mm. thickness. The masses are of various sizes, from 0·02 to 0·07 mm. in diameter, and are composed of small spherical micrococci. Cohn found them also in his (Cohn's) nourishing fluid (see Chapter II. A. 7), where they produce the peculiar smell of cheese. They are capable of changing acid nourishing material into alkaline. Cohn called the organism *ascococcus Billrothi.*

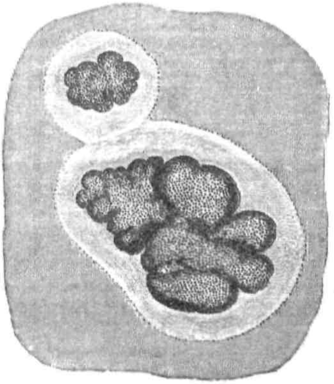

FIG. 17.—ASCOCOCCUS BILLROTHI (AFTER COHN).

Sarcinæ Ventriculi.—Goodsir was the first to describe in the vomit of some patients, peculiar groups of four cubical cells, with rounded edges, and closely placed against one another. These *sarcinæ ventriculi* are of a greenish or reddish colour. The diameter of the individual cells is about 0·004 mm. They are found in the contents of the stomach of man and brutes in health and disease, where the groups of four cells form smaller and larger aggregations. Occasionally small sarcinæ occur on boiled potatoes, egg albumen, and gelatine exposed to the air. These sarcinæ are considerably

smaller than the sarcina ventriculi, and when in large quantities have a yellowish tinge. Like the sarcina ventriculi they are in groups of four, and these again occur in larger or smaller aggregations and zooglœa. I have cultivated them successfully through many generations in pork broth, beef broth, mixture of gelatine and broth, at ordinary temperatures and in the incubator; more easily however at ordinary temperatures.

(*d*) *Pathogenic micrococci.*—Many of these are associated with disease. In the pus of open wounds,[1] and in that of closed abscesses, occur micrococci, singly, in dumb-bells, and in colonies or short chains,[2] but there are certain acute inflammations, *e.g.* that produced by subcutaneous injection of turpentine, the pus of which does not contain micrococci or any other organism.[3]

The secretion of open ulcers, such as occur in ordinary acute inflammations of the skin and mucous membranes, in ulcerations of the throat due to scarlatina, in every ulceration of the intestinal mucous membrane, in the lymph of the vesicles of the skin and mucous membranes of the mouth occurring in various kinds of inflammations, there are almost always present micrococci in dumb-bells, often also in beautiful chains. In the ulcers and abscesses they often form continuous masses, *i.e.* zooglœa, encroaching upon the tissue of the base of the ulcer. To this category belong the minute micrococci (about 0·0005 mm. in diameter) which Klebs described as microsporon septicum, found in and around wounds. The spread of purulent inflammation in

W. Cheyne, *Path. Trans.* vol. xxx.

[2] Ogston, "Micrococcus in Acute Abscesses," *Brit. Med. Journ.* March 12, 1881.

[3] Uzkoff, *Virchow's Archiv*, vol. 86, i. p. 150.

connective tissues and in parenchymatous organs is often, if micrococci are present in the original focus, associated with a corresponding spreading of the micrococci; these easily grow into all the spaces and crevices of the tissues, but whether this spreading of the micrococci is merely of secondary importance, *i.e.* concomitant with or subsequent to the spreading of the inflammation, or whether it is the primary cause as some assume, is not clear, and requires definite experimental proof.

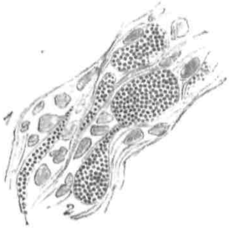

FIG. 18.—FROM THE BASE OF AN ULCER OF THE MUCOUS MEMBRANE OF THE LARNYX IN A CHILD THAT DIED OF ACUTE SCARLATINA.

1. Nuclei and fibres of the tissue.
2. Zooglœa of micrococci.

In all cases of diarrhœa the secretions of the bowels swarm with micrococci. In typhoid fever, clumps of micrococci may be found very extensively on the ulcerations of the bowels, and in the mucous membrane surrounding the ulcerations, and may be even traced into the mesenteric glands and the spleen.[1]

In dead tissues within the living body, such as occur after embolism, and in the case of various infectious maladies, micrococci may be found in colonies, *i.e.* as zooglœa,

[1] Klein, *Reports of the Medical Officer*, 1876. Letzerich, Sokoloff, Fischel, &c.

in the blood-vessels and in the parts around. The same holds good for the disseminated abscesses and necroses occurring in connexion with surgical pyæmia. In this malady masses of micrococci have been found in many of the affected organs.[1]

Wassilieff[2] has shown that these micrococci only occur after the death of the tissue or tissues, that in these they may multiply so as to form extensive colonies, and that therefore the presence of these micrococci is only a secondary phenomenon.

FIG. 19.—CAPILLARY BLOOD-VESSELS OF NECROTIC MASSES FROM THE LIVER OF A MOUSE. THE CAPILLARIES ARE DISTENDED BY, AND FILLED WITH ZOOGLŒA OF MICROCOCCI.

In pneumonia accompanying certain infectious maladies, *e.g.* typhoid fever, tuberculosis, and even in severe catarrhal pneumonia, large masses of micrococci may occur in the air-cells. In those cases where lobules and whole lobes become transformed into solid structures—grey hepatisation—masses of micrococci may be found in the air-cells, and even growing into the blood-vessels in which stasis had set in. Such is the case in pleuro-pneumonia of cattle and in the pneumonia of swine fever. Pasteur has cultivated micrococci which

[1] "Report of the Committee of the Pathological Society," *Path. Trans.* vol. xxx.
[2] *Centralb. f. d. med. Wiss.* No. 52, 1881.

were said to occur in the blood of pigs affected with the disease known in France as *rouget*, and which Pasteur considered identical with the disease known in this country as swine fever or swine plague. Pasteur asserts also that with these micrococci artificially cultivated he has reproduced the disease in swine. It appears, however, from an investigation by Schütz[1] that *rouget* is identical with the disease known in Germany as erysipelas of swine, and that this disease is caused not by a micrococcus at all, but by a small bacillus. This disease differs altogether from swine fever in its symptoms and course. Compare also Chapter XI. "Bacillus of Swine Fever."

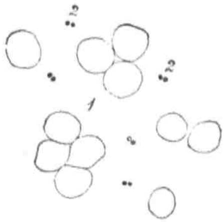

FIG. 20.—FROM A PREPARATION OF THE BLOOD OF A CHILD ILL WITH INFANTILE DIARRHŒA.

1. Blood-discs.
2. Dumb-bells of micrococci.

Micrococci occur always normally in large quantities in the fluids (saliva and mucus, &c.) of the nasal and oral cavities, pharynx, larynx, and trachea; they are derived no doubt from the atmosphere. On the papillæ filiformes of the tongue they form in some cases large masses.[2] Pasteur[3] has inoculated rabbits with the saliva of a child that suffered from hydrophobia, and having cultivated artificially the

[1] *Arbeiten aus d. k. Gesundheitsamte*, Berlin, 1885.
[2] Butlin, "Fur of the Tongue," *Proc. Roy. Soc.* 1880.
[3] *Comptes Rendus*, xlii.

micrococci present in this saliva, thought to have discovered that a micrococcus (*microbe spéciale*)[1] is the cause of hydrophobia. That saliva of the healthy dog and of man inoculated subcutaneously into rabbits sometimes produces death in these animals (Senator) had entirely escaped his notice, and Sternberg[2] has proved this in an extensive series of experiments. His own saliva proved sometimes fatal to rabbits. They die of what is called septicæmia, and Sternberg thinks it due to the micrococci; but this is not to be considered as satisfactorily proved.

All these micrococci stand therefore in no definite causal relation to the respective maladies, but are probably only of secondary importance.

The following micrococci are considered to stand in an intimate relation to specific diseases:—

1. *Micrococcus variolæ et vacciniæ.*—Chauveau[3] was the first to prove experimentally that in vaccinia and in variola the active principle is a particulate non-diffusible substance. Burdon Sanderson confirmed and extended this.[4] Cohn[5] proved that the lymph of vaccinia and variola contains numerous micrococci. Weigert[6] showed for human small-pox, Klein[7] for sheep-pox, that the lymphatics of the skin in the region of the pock are filled with micrococci, and

[1] It is not quite clear whether this *microbe spéciale* is a dumb-bell micrococcus or a bacterium termo; it is quite possible that it is the latter, viz. a rod constricted in the middle. If so, it would appear identical with the bacterium that produces Davaine's septicæmia in rabbits (see Chapter VIII.).

[2] *Bulletin of the National Board of Health, U.S.A.* April 30, 1881.

[3] *Comptes Rendus*, 1868.

[4] *Reports on the Intimate Pathology of Contagion.*

[5] *Virchow's Archiv*, 1872. Keber, Hallier, Zürn.

[6] *Med. Centralb.* 1871.

[7] *Phil. Trans.* 1874.

Pohl-Pincus[1] traced their passage through the epidermis at the point of vaccination in the calf. The micrococci are very minute, according to Cohn's estimate 0·0005 mm. and less in diameter, single or in dumb-bells, or in shorter or longer chains, or in small groups. When cultivated on the warm

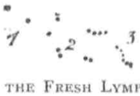

FIG. 21.—MICROCOCCUS IN THE FRESH LYMPH OF HUMAN SMALL-POX.
1. Singly.
2. In dumb-bells.
3. In short chains.

stage, they form very long chains and colonies. In connexion with this it must be mentioned, as stated on a former page, that similar micrococci occur also in the fluid contents of vesicles in the skin produced by various non-infective inflammations. To make it sure that the micrococci are the

FIG. 22.—LYMPHATIC VESSEL FROM THE SKIN OF A POCK IN SHEEP-POX.
The vessel is filled with micrococci.

active principle, *i.e.* the *causa morbi*, it would be necessary to artificially cultivate them through several generations, and then, by re-inoculating them, to reproduce the disease. This essential link in the evidence is, however, still wanting.[2]

[1] *Vaccination*, Berlin, 1882.
[2] See the prize announcement of the Grocers' Company, London, 1883.

2. *Micrococcus erysipelatosus.*—The micrococcus is very minute, smaller than that of vaccinia. Lukomsky[1] showed that, at the margin of an erysipelatous zone, that is the part where the disease is progressing and marked by the characteristic redness and swelling, the lymphatics of the skin are filled with zooglœa of micrococci, and the injection of these vessels keeps pace with the progress of the erysipelatous process. Orth[2] cultivated these micrococci artificially, and with such cultures produced by inoculation erysipelas in rabbits. Fehleisen[3] placed this beyond any doubt, inasmuch as he produced successive cultures of these micrococci (derived from the lymphatics of erysipelatous human skin), and then by re-inoculation produced the disease not only in rabbits but also in man. Fehleisen found the micrococci only in the lymphatics of the affected parts, and these he cultivated artificially for fourteen generations—which it took two months to do—on peptonised meat-extract gelatine, and solid serum. The micrococci form a whitish film on the top of the nourishing material, and when inoculated into the skin (ear) of rabbits, a characteristic erysipelatous rash makes its appearance after from thirty-six to forty-eight hours, and spreads to the root of the ear, and further on to the head and neck. The animals do not, however, die from it. In the human subject he produced typical erysipelas after inoculation with the pure cultivated micrococcus in fifteen to sixty hours. These inoculations were justifiable because they were undertaken with a view to cure certain tumours. Thus one case of lupus, one case of cancer, one case of sarcoma, were considerably affected, and to the good of the patient.

[1] *Virchow's Archiv*, vol. 60.
[2] *Archiv f. exp. Path.* Bd. i. 1874.
[3] *Die Aetiologie d. Erysipels*, Berlin, 1883.

Fehleisen also in several instances carried out second inoculation within a few months. The also found that a 3 per cent. solution of carb a 1 per cent. solution of corrosive sublimate vitality of this micrococcus.

3. *Micrococcus diphtheriticus.*—Buhl, Hüte had shown that in diphtheria the memb micrococci. Oertel[1] found this micrococ numbers, not only in the diphtheritic memb organs of the throat and in their neighbourho in the surrounding lymphatics, but also in the general circulation, in the kidney, and in the r

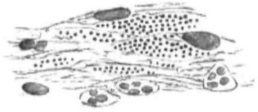

FIG. 23.—PORTION OF A DIPHTHERITIC MEMBRA Numerous micrococci present.

micrococci are about 0·00035 to 0·001 mm. in slightly oval, occur singly or in dumb-bells or in they form also continuous masses of zooglœa of spherical or cylindrical clumps, and a penetrate and destroy the surrounding co muscular tissues. In severe cases they are fo up the capillaries of the glomeruli and the urini of the kidney.

Besides micrococci there occur in the diph branes also other (rod-shaped) bacteria, but these

[1] "Experim. Unters. über Diphtherie," *Deutsches Med.* Bd. viii. 1871.

only accessory.[1] Cultivations and inoculations with pure cultivations of this micrococcus are still wanting.

4. *Micrococcus pneumoniæ.*—In acute croupous pneumonia there occur in the affected lungs large numbers of micrococci; Klebs, Eberth, Koch, Leyden, and others have seen them, but Friedländer[2] first pointed out their constant occurrence. According to this observer they are oval, each possessed of a capsule, about 0·001 mm. long, and occur in the sputum singly, but especially as dumb-bells or diplococci, as chains, and as zooglœa. Ziehl[3] found them in very large crowds in the sputum, giving to this in the early stages the peculiar characteristic brownish "prune-juice" tint. But this statement cannot be correct, since this tint may be very pronounced although the sputum contains only a limited number of the micrococci. According to this observer, they are very numerous only in the beginning of the illness; after the critical stage they decrease in numbers.

Griffini and Cambria saw the micrococci also in the blood. Salvioli found that their number increases after the third day; on the ninth or tenth day they quite disappear.

G. Giles[4] found them in many cases of pneumonia (in India), both in the sputum and in the blood. Cultivations on boiled potato yielded good crops. These cultivated micrococci injected into the subcutaneous tissue of rabbits produced pneumonia.

Salvioli and Zäslein[5] cultivated the micrococci (derived

[1] Compare also Klebs, *Archiv f. exp. Path.* iv.; Letzerich, *Virchow's Archiv*, vol. 68; Nassiloff, *ibid.* vol. 50; Eberth, *Zur Kenntniss d. bact. Mycosen*, 1872; Wood and Formad, *Report of National Board of Health, U.S.A.* 1882.

[2] *Virchow's Archiv*, vol. 87.

[3] *Centralb. f. med. Wiss.* No. 25, 1883.

[4] *Brit. Med. Journ.* July 7, 1883.

[5] *Centralb. f. med. Wiss.* No. 41, 1883.

from the blood) in meat broth, meat extract solution, &c., at 37° to 39° C., and obtained good crops of them, with which they produced by inoculation in seven rabbits and six white rats, typical pneumonia yielding the characteristic micrococci. These micrococci stain best in a mixture of Bismarck brown and methyl violet, but they stain also very well in gentian violet.

Quite recently Friedländer and Frobenius [1] cultivated the micrococci in nutritive gelatine, and obtained good crops. The shape of such growths is nail-like, consisting of a vertical streak, the nail-pin corresponding to the channel of inoculation, and in connexion with this is a disc-shaped patch—the nail-head—extending on the surface of the gelatine. Inoculations of these capsulated *pneumococci* into the lung tissue of rabbits produced no result, into that of mice produced pneumonia and pleurisy after twenty-four to forty-eight hours. In guinea-pigs the results were not so decisive. About half of the animals escaped, the others died with dyspnœa, the blood, lungs, and pleural exudations containing the same pneumococci. From my own observations I cannot accept these statements without qualification, for I find: That even in typical cases of croupous pneumonia of man, the micrococci may be absent or may be only very scarce even between the third and ninth day; that typical sputum of croupous pneumonia does not in many instances produce disease in animals on inoculation; and that the disease produced in rabbits and mice is of the nature of septicæmia, due to a specific septicæmic micrococcus not necessarily always present in the sputum and lungs of human croupous pneumonia.

It seems therefore clear, that when sputum produces on inoculation disease and death of rodents, this is due to

[1] *Berichte d. physiolog. Gesellschaft in Berlin*, Nov. 9, 1883.

the accidental admixture of a capsulated micrococcus, which, according to Dr. Sternberg of Baltimore, is probably identical with the one occasionally present in the fluid of the mouth even of healthy persons. Capsulated micrococci, identical in morphological respects and mode of growth in artificial

FIG. 24.—FROM A SECTION THROUGH THE HUMAN LUNG IN ACUTE CROUPOUS PNEUMONIA.

Three capillary vessels filled with blood are seen surrounding an alveolus which is filled with fibrin and blood corpuscles, amongst them chains of micrococci. Magnifying power 700. (Stained with gentian violet.)

cultivations, occur in other conditions not connected with croupous pneumonia.

Bruylants and Verriers[1] assert that they have successfully cultivated the micrococci of pleuro-pneumonia of cattle.

[1] *Bull. de l'Acad. Belg.* 1880.

More recently [1] T. Poels and Dr. W. Nolen, in Rotterdam, assert that they have ascertained that in pleuro-pneumonia of

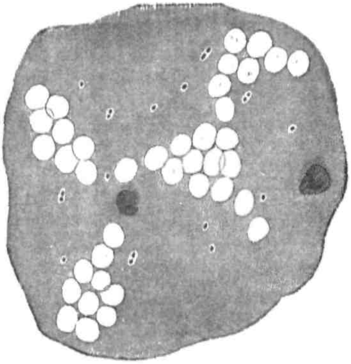

FIG. 25.—FROM A PREPARATION OF BLOOD OF RABBIT DEAD AFTER INOCULATION WITH SPUTUM OF ACUTE CROUPOUS PNEUMONIA.

Weigert-Koch method, stained with gentian violet. Numbers of blood discs, between them oval micrococci, surrounded by hyaline capsules.

Magnifying power 700.

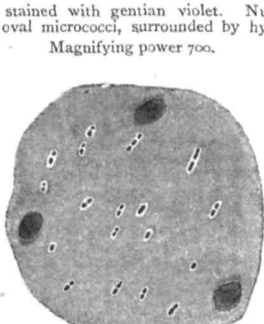

FIG. 26.—FROM A PREPARATION OF PLEURAL EXUDATION OF A MOUSE DEAD AFTER INOCULATION WITH BLOOD OF RABBIT MENTIONED IN PRECEDING FIGURE.

Magnifying power 700.

cattle the pulmonary exudations contain micrococci, which in their morphology and mode of growth in artificial cultures

[1] *Centralb. f. d. med. Wiss.* No. 9, 1884.

are identical with the micrococci of human pneumonia. And they further assert that artificial cultures of the micrococci derived either from human pneumonia or from pleuro-pneumonia of cattle, produce in cattle the typical pleuro-pneumonia. From my own observations I have reason to doubt the accuracy of these statements.

5. *Micrococcus gonorrhœæ.*—Micrococci have been found in the pus of gonorrhœa. Neisser,[1] and later Bokai and Finkelstein,[2] described them as spherical organisms of about

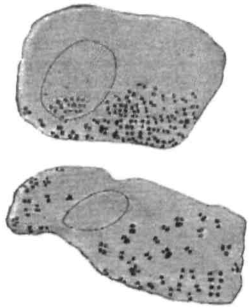

FIG. 27.—TWO LARGE SCALY EPITHELIAL CELLS OF GONORRHŒAL PUS.
The epithelial cells are covered with micrococci, chiefly in dumb-bells, some in sarcina form.

0·008 mm. diameter, generally forming dumb-bells, or sarcina-like colonies of four. Several such groups form a zooglœa. They adhere to the pus-corpuscles and epithelial cells. They stain easily and well in methyl violet and gentian violet. Bockhart[3] has succeeded in artificially cultivating these micrococci, and in producing with them the disease by

[1] *Centralb. f. d. med. Wiss.* No. 28, 1879.
[2] *Prager med. chir. Presse*, May, 1880.
[3] *Sitzungsberichte der phys.-med. Gesellsch. in Wurzburg*, Sept. 1882.

inoculation. The pathogenic character of these micrococci is denied by Sternberg.[1]

Aufrecht [2] reports the case of an infant twelve days old who died with suppuration of the umbilical vein and liver. The liver cells and the interlobular tissue were crowded with micrococci (shown in sections by means of a 2 per cent. watery solution of Bismarck brown). These micrococci corresponded in size to the micrococcus gonorrhœa, and he thinks it probable that they were derived from the vagina of the mother; during birth they might have got into the umbilical vein, there caused inflammation, and thence passed into the liver.

6. *Micrococcus endocarditicus.*—Micrococci in the form of zooglœa have been seen in *endocarditis ulcerosa.* They sometimes form plugs in the blood-vessels of the muscular tissue of the heart (Heiberg,[3] Maier,[4] Eberth,[5] Köster,[6] Klebs [7]). Heiberg saw the micrococci forming chains in the muscle of the heart, in the detritus of the ulcerations of the endocardium, in the plugs in the vessels of the spleen and kidney.

7. *Micrococcus scarlatinæ.*—In *scarlatina* Coze and Feltz [8] described micrococci as occurring in the blood; as I have mentioned above, I have seen them in the ulcerations of the throat,[9] and quite recently Pohl-Pincus [10] described very minute micrococci adhering to the scales of the desquamating epidermis in scarlatina. They form small colonies, and stain

[1] *Medical News*, No. 16, 1884.
[2] *Centralb. f. d. med. Wiss.* No. 16, 1883.
[3] *Virchow's Archiv*, vol. 56.
[4] *Ibid.* vol. 62.
[5] *Ibid.* vol. 57.
[6] *Ibid.* vol. 72.
[7] *Archiv f. exp. Path.* Bd. 9.
[8] *Malad. infect*
[9] *Report of the Medical Officer of the Privy Council for* 1876.
[10] *Centralb. f. d. med. Wiss.* No. 36, 1883.

violet with a saturated solution of methyl violet. Their diameter is very small, only about 0·0005 mm. The same micrococci were noticed by Pohl-Pincus in the throat-discharge.[1] See Bizzozero's statements mentioned on p. 61.

8. In *cattle plague*, also called rinderpest, micrococci have been found in the lymphatic glands by Klebs (1872) and by Semmer in the blood and lymphatic glands (1874 and 1881). In conjunction with Archangelski,[2] Semmer cultivated the micrococci obtained from the lymphatic glands of a sheep dead of inoculated rinderpest, in beef broth, in meat-extract solution, and in mixture of broth, peptone, and gelatine at 37° to 39° C. The micrococci grew very copiously as zooglœa and in chains. With these micrococci (of a first transfer or cultivation) a calf was inoculated, and died after seven days from rinderpest. The cultures when transferred lose gradually their virulence from one generation to the next, but animals (sheep) inoculated with these are protected against further virulent disease. Further, cultures exposed for an hour to a temperature of 46° or 47° C. become greatly attenuated in their action, and sheep inoculated with virus thus attenuated are protected against virulent material. Temperatures of − 10° to − 20° C. annihilate the activity of rinderpest organisms. The specific nature of these micrococci of rinderpest cannot, however, be considered at all established as in the case of those mentioned above, *e.g.* the micrococci of erysipelas.

9. In *puerperal fever* micrococci have been found in the form of zooglœa by Heiberg,[3] in all affected organs—endocardium, lung, spleen, cornea, in a case of panophthalmitis

[1] Seen already by McKendrick, *Brit. Med. Journ.* 1872.
[2] *Centralb. f. d. med. Wiss.* No. 18, 1883. [3] Leipzig, 1873.

puerperalis, and in the kidney, forming casts in the uriniferous tubules and emboli in the blood-vessels. Laffler[1] found zooglœa and chains of micrococci in two cases of puerperal fever associated with brain-softening. In both cases emboli, due to micrococci, were found in the surroundings of the softened part of the brain. Emboli of micrococci were also here found in the vessels of the kidney.

10. In *pernicious anæmia* Frankenhäuser[2] described the occurrence of micrococci (?) in the blood of pregnant women suffering from this anæmia, not uncommon in Zürich. These micrococci were very large, about one-tenth of the broad diameter of a red blood-corpuscle, and some were provided with a flagellum (?). Some were divided in two. In the blood of the liver they occurred in large numbers. Frankenhäuser's description makes it very difficult exactly to understand what he saw. He also states that these micrococci were probably derived from decayed teeth, from which all his patients suffered.

Eppinger[3] described micrococci as occurring in *acute yellow atrophy* of the liver.

11. In the *syphilitic mucous patches* of several patients Aufrecht found a micrococcus, forming generally dumb-bells and staining very deeply in fuchsin.[4] Birch-Hirschfeld[5] confirmed this.

12. *Micrococcus of acute infectious osteomyelitis.*—Dr. Becker has made, in the laboratory of the Berlin Imperial Sanitary

[1] *Breslauer ärztl. Zeitschrift*, 1880.
[2] *Centralb. f. d. med. Wiss.* No. 4, 1883.
[3] *Prager Viertelj.* 1875.
[4] *Centralb. f. d. med. Wiss.* No. 13, 1881.
[5] *Ibid.* No. 44, 1882.

Office, a series of important experiments on the micro-organisms discovered by Schüller and Rosenbach. He collected pus from five cases of acute osteomyelitis in which the abscesses had not been opened, and cultivated the micrococci contained in it on sterilised potatoes, coagulated serum, and gelatine-peptone. In the latter case, the pus was introduced by means of needles into the mass, which was then kept at the temperature of the room during three or five days. After that time, the punctures made by the needles assumed the appearance of white streaks, around which the gelatine gradually liquefied and took an orange colour. After a few days more, the mass gave out a smell like sour paste, and the microscope revealed the presence of large numbers of micrococci, having the same appearance as those found in the pus. A small quantity of the mass was mixed with sterilised water and injected into the peritoneal cavity of some animals; they died in a very short time of acute peritonitis. The same fluid injected into the jugular vein caused acute septicæmia and death; but nothing abnormal was found in the bones in either case. Dr. Becker then injected a small quantity of the same fluid into the jugular veins of fifteen rabbits, after having, some days before, fractured or bruised the bone of one of the hind legs. At the end of the first week a swelling was formed at the seat of the bruise or fracture, the animals lost flesh, and died after a few days. On dissection, large abscesses were found around and in the bones, and in several cases metastatic abscesses had formed in the lungs and kidneys. Numerous colonies of micrococci were discovered in the blood and pus of the animals upon which the experiments were made. This micrococcus is identical with the micrococcus pyogenes aureus of Rosenbach. When growing on Agar-Agar mixture it forms flat irregular patches of an orange colour.

13. Koch[1] described various kinds of micrococci intimately connected with certain destructive (pyæmic) processes in mice and rabbits. (*a*) Micrococcus of *progressive necrosis* in mice. Injecting into the ear of mice—white mice, or better, field mice—putrid fluids, he observed a necrosis of the tissues of the ear (skin, cartilage) starting from the point of inoculation and gradually spreading on to the surrounding parts and killing the animal in about three days. As far as the necrosis reaches, the tissue is crowded with micrococci, chiefly in the form of chains and zooglœa. The individual cells are spherical, of about 0·0005 mm. in diameter. I may mention that I have found a somewhat different micrococcus virulently active on mice. I have inoculated a number of white mice subcutaneously in the tail with a small micrococcus cultivated through several generations, and apparently derived from an artificial cultivation in pork broth, but due to accidental contamination. These micrococci, after having been cultivated in pork broth through several generations, were used in infinitesimal doses for the inoculation of the above mice. In two instances I have seen that the inoculation was followed after two or three days by purulent inflammation at the seat of inoculation, but apparently not spreading beyond it. But as time went on inflammation and abscess in the lungs set in and the animals died after about a week. On making longitudinal sections through the tail, it was found that in most of the lymph-spaces and lymph-vessels of all parts of the cutis and subcutaneous tissue, far away from the seat of inflammation, there were densely crowded masses of the same minute micrococci as were used for inoculation. And these crowds of micrococci could be traced to the seat of inflammation,

[1] *Untersuchungen über die Aetiologie d. Wundinfections-Krankheiten*, Leipzig, 1878.

where they extended amongst the inflammatory products in great masses. The abscesses in the lungs were filled with the same micrococci. Inoculated into the skin of fresh mice, it again produced death by pyæmia. This micrococcus may therefore be called the *micrococcus pyæmiæ* of mice. (*b*) Micrococcus causing *abscesses* in rabbits. Putrid blood injected into the subcutaneous tissue of the rabbit often produces suppurative abscess which, spreading, kills the animal in about twelve days. In the wall of the abscess

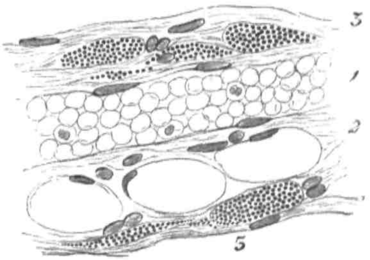

FIG. 28.—FROM A SECTION THROUGH THE TAIL OF A MOUSE INOCULATED INTO THE SUBCUTANEOUS TISSUE OF THE TAIL WITH ARTIFICIALLY CULTIVATED MICROCOCCUS.

The part here illustrated is a good distance from the ulceration.

1. A capillary blood-vessel filled with blood-corpuscles.
2. Fat cells.
3. Groups of micrococci filling the lymph-spaces of the connective tissue.

are found continuous masses of zooglœa of micrococci. The pus is infectious. The micrococci are spherical, and of a very minute size, measuring only about 0·00015 mm. in diameter. (*c*) Micrococcus causing *pyæmia* in rabbits. Skin of a mouse was macerated in distilled water for two days, and of this fluid a hypodermic syringeful was injected under the skin of the back of a rabbit. After two days the animal began to lose flesh and died after 105 hours. Purulent infiltration spread from the seat of inoculation into

the subcutaneous tissue; peritonitis; spleen much enlarged; slight pneumonia. A hypodermic syringeful of the blood of this animal was injected under the skin of a second rabbit, and this died after forty hours. Post-mortem examination showed the same lesions as in the first case. In

FIG. 29.—FROM A PYOGENIC MEMBRANE COVERING THE SEROUS COAT OF THE INTESTINE OF A RABBIT DEAD OF PYÆMIA.

1. A large oval nucleus, probably the nucleus of a detached endothelial cell.
2. A pus corpuscle.

The rest of the pyogenic membrane is beset with small micrococci.

FIG. 30.—PYÆMIA OF RABBIT.

Blood of spleen. Between red blood-discs three dumb-bells and two single micrococci are shown. (Gentian violet staining.)

[The micrococci as here represented are somewhat too large.]

the blood-vessels of the affected parts were present micrococci, single, as dumb-bells, and in zooglœa; they were spherical, about 0·00025 mm. in diameter. (*d*) Micrococcus causing *septicæmia* in rabbits. An infusion of meat was

FIG. 31.—OVAL MICROCOCCI FROM THE BLOOD-VESSEL OF THE SPLEEN OF A RABBIT, DEAD OF KOCH'S SEPTICÆMIA.

prepared; this was left to putrefy, and of this fluid a quantity was injected under the skin of the back in two cases. Extensive gangrene with much œdematous exudation followed, and death ensued in two days and a-half. The blood, the

capillaries of the kidney, and the enlarged spleen, contained numerous oval micrococci. Two drops of the œdematous exudation-fluid were injected under the skin of the back of another rabbit. Death followed in twenty-two hours. There

FIG. 32.—CULTIVATION OF FOOT AND MOUTH MICROCOCCUS ON NUTRITIVE GELATINE, AFTER SEVERAL WEEKS' GROWTH.

was no gangrene here; but œdema was present, spreading from the seat of the inoculation. Sub-serous hæmorrhages appeared in the intestines; and minute hæmorrhages were also present in the œdematous tissue and in the muscles of

the thigh and abdomen. The œdematous fluid, the cutaneous veins, the capillaries in the kidney, especially those of the glomeruli, in the lung, and in the spleen, contained numerous oval micrococci, singly, as dumb-bells, and in zooglœa. The micrococci measured about 0·0008 to 0·001 mm. in their long diameter. These micrococci (taken with the blood) produced in another rabbit and in a mouse the same fatal disease.

14. *Micrococcus bombycis* (Microzyma bombycis, Béchamp).—Oval micrococci, of about 0·0015 mm. in length, present in large numbers, singly, and as dumb-bells and chains

FIG. 33.—SAME CULTIVATION AS IN FIG. 32 SEEN UNDER A LENS.

(straight or curved), in the contents of the alimentary canal and in the gastric fluid of silkworms dead of the "maladie de mortsblancs, *flacherie*."—*Micrococcus ovatus*, Nosema bombycis. Present in large numbers in the blood and organs, ova included, of silkworms affected with the disease called "maladie des corpuscles," "pébrine," or Cornalia's disease. Cornalia first saw them, afterwards Lebert and Nägeli. Pasteur proved definitely that ingestion as well as inoculation of the silkworms with the micrococci produces the disease. The micrococci are comparatively large, 0·003 to 0·004 mm. long, 0·002 mm. broad; they are very bright and occur singly, or in dumb-bells, or in small groups.

15. *Micrococcus of foot-and-mouth disease.*—In the vesicles of sheep ill with this disease I find a micrococcus, singly, in dumb-bells, and in curved chains. It stains well with the ordinary aniline dyes. It grows well in milk, in alkaline peptone broth, in nutrient gelatine and in Agar-Agar mixture. Growing on solid material its growth, besides being extremely slow, is very characteristic, it forms a film composed of minute granules or droplets, closely placed side by side, but not confluent. It is highly sensitive towards antiseptics. It does not liquefy nutritive gelatine, and in liquids does not form a pellicle, but nevertheless when grown on solids, its growth remains limited to the surface. It does not curdle milk, although it turns the reaction of this latter slightly but distinctly acid.

CHAPTER VIII.

BACTERIUM (*Microbacterium*, Cohn).

By this name Cohn [1] designates a class of minute schizomycetes, which are slightly elongated and oval, or short and cylindrical, with rounded ends. They divide by fission, like the micrococci, the individuals elongating and becoming constricted in the middle. They are capable of spontaneous locomotion, being possessed of a flagellum at one or both ends, with which they perform active spinning and darting movements (Dallinger). Engelmann has shown that these movements are only possible in the presence of oxygen. Bacteria are found also as dumb-bells, *i.e.* in the act of dividing, and then appear as rods constricted in the middle. Occasionally, after rapid division, several remain connected, thereby forming a short chain. In this state the terminal elements are flagellate. Bacteria, like micrococci, are capable of forming zoogl[oe]a, the interstitial gelatinous substance being, as a rule, more copious than in the zoogl[oe]a of micrococci. In this state they form pellicles, in which the elements are without flagella; but from the margin of the pellicles one constantly sees elements separating, becoming flagellate, and moving away. In some species

[1] *Biologie d. Pflanzen*, ii. (1872), p. 167.

the zoogl≈ìa is dendritically ramified (*Zoogl√¶a ramigera*, Itzigsohn), as seen on the surface of fluids containing decomposing alg√¶.

1. *Septic Bacteria.*—With Cohn we distinguish two kinds :—*Bacterium termo* and *Bacterium lineola.*

(*a*) *Bacterium termo.*—The elements are short and cylindrical, about 0·0015 mm. long, a third less in breadth, and appear generally as dumb-bells. They are common in putrefying fluids, indeed they form the essential cause or ferment of decomposition, being the true saprogenous ferment (Cohn). They are invested in a thick membrane,

FIG. 34.—BACTERIUM TERMO FROM AN ARTIFICIAL CULTURE.

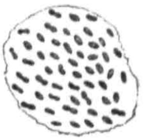

FIG. 35.—ZOOGL≈íA OF BACTERIUM TERMO.

and are flagellate. With the end of putrefaction they disappear. They grow well in Cohn's nourishing fluid, and I have found them as constant inhabitants of unfiltered distilled water in the laboratory ; so much so that with a drop of this water I am always able to start a copious growth of bacterium termo in pork broth, Agar-Agar, &c. When cultivated in the incubator at 32° to 36° C. in suitable nourishing material (pork broth, chicken broth,) they produce a uniform turbidity, and after several days an attempt at a pellicle, the whole nourishing fluid becoming thicker. But after from several days to a few weeks the cultures die, a fact which distinguishes them from all other

bacteria. Growing in solid Agar-Agar and peptone mixture, they produce an imperfect liquefaction, numerous gas bubbles appearing in the material.

(*b*) *Bacterium lineola* (*Vibrio lineola*, Ehrenberg, Dujardin), differs from bacterium termo in being longer and thicker. The cells are about 0·003 to 0·005 mm. long, about 0·0015 mm. thick. They occur in well-water and stagnant water, where no distinct putrefaction is going on, and form zooglœa, and pellicles, on the surface of potatoes and various infusions.

FIG. 36.—BACTERIUM LINEOLA.

FIG. 37.—BACTERIUM LACTIS.

2. *Zymogenic Bacteria.*—Two kinds are known: *Bacterium lactis* and *Bacterium aceti*.

(*a*) *Bacterium lactis.*—According to Pasteur, these bacteria are about 0·0015 to 0·003 mm. long, constricted in the centre; they form short chains, or even zooglœa, and they are motile. They produce the lactic acid fermentation, in the course of which lactic sugar is transformed into lactic acid; they are anaërobic. Lister,[1] by means of pure cultures, established experimentally their causal relation to the lactic fermentation or souring of milk.

(*c*) *Bacterium aceti* (*Mycoderma aceti*) is a little smaller than bacterium lactis, being about 0·0015 mm. in length, and often forms chains, and also pellicles, on the surface of the fluid; it is motile. Pasteur maintains that it is the ferment of the acetic acid fermentation. Cohn[2] found it in

[1] *Pathological Soc. Transactions*, 1878.
[2] *Biol. d. Pflanzen*, ii. p. 173.

enormous masses in beer that had become sour; it forms dumb-bells, seldom chains of four, and sometimes a pellicle on the surface. Pure cultivations have not been made with it, and before deciding whether it is the real cause of the acetic acid fermentation, experiments with such pure cultures, *i.e.* inoculations of alcoholic fluids with it, are required.

3. *Pigment Bacteria.*—Two kinds have been described: *Bacterium xanthinum* and *Bacterium aeruginosum.*

(*a*) *Bacterium xanthinum*[1] is a bacterium about 0·007 to 0·01 mm. long, motile, single, also in dumb-bells, or short chains. It produces the yellow colour of yellow milk. Its pigment is soluble in water, and insoluble in alcohol or ether. When introduced into boiled milk of neutral reaction, it multiplies with great rapidity; the milk coagulates after twenty-four hours; it is soon teeming with them and turns yellow. The reaction of the yellow milk is at first acid, but soon becomes alkaline, and the alkalinity gradually increases.

(*b*) *Bacterium aeruginosum.*—In green pus Schrœter discovered a bacterium, *Bacterium aeruginosum.*[2] The pigment is greenish, and not lodged in the cells themselves; it is easily diffusible.

4. *Pathogenic Bacteria.*—Three kinds are described; the bacteria of Koch's septicæmia, of Davaine's septicæmia, and of fowl-cholera.

(*a*) *Bacterium septicæmiæ* (Koch). — By injecting into rabbits water from the rivulet Pauke, and from putrid mutton, Koch[3] succeeded in producing a rapidly fatal septicæmia,

[1] Schrœter, *Biol. d. Pflanzen*, ii. p. 120; *Vibrio synxanthus*, Ehrenberg.
[2] *Loc. cit.* p. 122.
[3] *Mitth. aus d. k. Gesundh.* 1881.

which was characterised by the following appearances :—The blood of all the organs contained very numerous bacteria, the spleen and lymphatic glands were enlarged, and the lungs congested; but there were no extravasations and no peritonitis. The smallest quantity of this blood inoculated into the skin or cornea of another rabbit produced after an incubation of ten to twelve hours distinct rise of temperature, and death after sixteen to twenty hours. The conditions after death were the same as above. Everywhere the blood contained the bacteria. They are rods somewhat pointed at both ends, measuring about 0·0014 mm. in length and 0·0006 mm. in breadth. When stained, they show at each end a deeply-tinted granule, the middle part remaining unstained; for this

FIG. 38.—BLOOD OF PIGEON, DEAD OF SEPTICÆMIA.
Four blood-discs and four bacteria-termo are shown.

reason they are easily mistaken for a diplococcus. Generally these rods occur singly; occasionally they form a chain of two, or more than three.

They have been cultivated successively in beef broth, blood serum, gelatine, and a mixture of gelatine and broth and peptone. The cultures have the same virulent properties as the original blood.

Mice, pigeons, fowls, and sparrows are also very susceptible to these bacteria; but guinea-pigs, dogs, and rats resist them successfully.

The microbe found by Pasteur in human saliva, which he cultivated, and with which he produced septicæmia in

rabbits, may perhaps be a bacterium identical with the above, but this is not definitely settled.

(*b*) *Bacterium of Davaine's septicæmia.*—This is a bacterium which was originally derived by Davaine[1] from putrid ox-blood in the warm season. Injected into rabbits it produced rapidly fatal septicæmia, of the same nature as in the case just mentioned, the blood teeming with a similar kind of bacterium as in Koch's septicæmia just described. The smallest quantity of the blood is again rapidly fatal in its action. It is distinguished from Koch's septicæmia in the rabbit by this, that Davaine's septicæmia is easily transmissible to guinea-pigs, but not to birds.

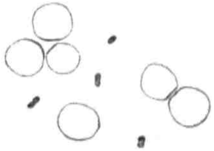

FIG. 29.—BLOOD OF RABBIT, DEAD OF DAVAINE'S SEPTICÆMIA.

Dowdeswell[2] has shown that when such blood is thoroughly sterilised (*i.e.* when the bacteria are killed), it has no longer any infective power. Davaine had first shown that the blood of rabbits dead of this form of septicæmia bears an enormous amount of dilution without the minutest quantity of it losing its pathogenic properties. Dowdeswell has shown that this is easily explained by the enormous number of bacteria present in every drop of the blood. But it has been shown by Gaffky and Dowdeswell that there is no increase in the virulence of the virus when it is passed

[1] *Bull. d. l'Acad. de Méd.* 1872.
[2] *Proceedings of the Royal Society*, No. 221, 1882.

through successive animals, as was maintained by Coze and Feltz.[1]

(*c*) *Bacterium of fowl-cholera* (*microbe du choléra des poules*). —Semmer, Toussaint, and Pasteur[2] have shown that this organism is present in large numbers in the blood and organs of fowls dead of this malady, which is chiefly characterised by the following symptoms:—The animals are somnolent, weak in their legs and wings, and they die under symptoms of extreme sopor. On post-mortem examination, hæmorrhage is found in the duodenum. The smallest quantity of the blood is infective. Pasteur successfully cultivated the bacteria in neutral chicken broth, at 25° to 35° C., and with it inoculated the fatal disease. The organism is probably a bacterium termo, very minute and slightly constricted in the middle, so that it appears of the shape of an 8. When cultures of this bacterium[3] are kept for some time (one, two, three or more months), their virulence becomes diminished or attenuated (owing, according to Pasteur, to the action of oxygen), and this diminution of virulence is in direct proportion to the time the culture is kept. The diminution or

[1] Strasburg, 1866; Paris, 1872.

[2] I place this here as a bacterium, but it is not quite decided, and not quite clear from Pasteur's description, whether the microbe is only a micrococcus dumb-bell, or a bacterium termo. Compare also Semmer (*Vergleichende Pathologie*, 1878), Perroncito (*Archiv f. wiss. u. pract. Thierheilk.* 1879. Toussaint (*Comptes Rendus*, xci. p. 301) considers the disease identical with Davaine's septicæmia. I am inclined to think that Pasteur has not used pure cultivations, but had the bacterium of fowl-cholera and an accidental micrococcus together. The latter would predominate as time passes on, so that after some days it would far outnumber the bacterium; and this is exactly what Pasteur's description suggests. He says that at first the microbe is rod-shaped, and after a few days it becomes a dumb-bell of micrococcus. The gradual attenuation by time of the virulence of Pasteur's cultures of the microbe of fowl-cholera may be due to the presence of this contaminating micrococcus.

[3] *Trans. of the International Med. Congress in London*, 1881, vol. i. p. 87.

attenuation shows itself in this—that according to the length of time the culture is kept, the number of animals killed by its inoculation gradually diminishes, and it ultimately ceases to kill at all. Each culture of diminished virulence transmits its attenuation to the next following culture (?). It is possible to obtain cultures of such a low degree of virulence that when inoculated into the skin of a fowl only a local effect is produced, a peculiar infiltration; but the animal survives, and is then protected or "vaccinated" against the more virulent material. But in order to produce this protection, it is necessary that the culture (*vaccine*) should be of the proper strength. If it does not produce a local effect it gives no protection.

In fresh cultures the bacterium is more in the shape of a rod, constricted in the middle; in cultures several days old it looks very much more like a dumb-bell of micrococcus (see note on previous page).

Babes[1] has found the bacteria in the tissues and blood-vessels of animals dead of the disease, both inoculated and epizootic, in the shape of rods of about 0·0015 to 0·002 mm. in length and about 0·00025 mm. in thickness; the ends always staining more deeply than the middle part.

[1] *Archives de Physiologie*, July 1883, p. 49.

CHAPTER IX.

BACILLUS (*Desmobacterium*, Cohn).

General Characters.—Bacilli are cylindrical or rod-shaped bacteria, which are rounded or square-cut at their extremities; they are longer in proportion to their thickness than bacterium termo, and divide by fission, forming straight, curved, or zigzag chains of two, four, six, or more elements. Many species of bacilli in suitable nourishing material grow by repeated division into longer or shorter chains of bacillus—filaments or *leptothrix*. These appear straight or wavy and twisted, isolated or in bundles; and although in the fresh condition they appear of a homogeneous aspect, when suitably prepared, as by drying and staining with aniline dyes, they show themselves composed of shorter or longer cubical, cylindrical, or rod-shaped protoplasmic elements, contained in linear series within a general hyaline sheath: between many of the elements is a fine transverse septum. The isolated bacilli are likewise composed of a membrane and protoplasmic contents. These latter appear homogeneous or finely granular, and when stained with aniline, absorb the dye very easily and retain it better and longer than the sheath. According to the stage and the rapidity of their growth, the bacilli vary much in length; this is the case not only with

the single bacilli and short chains, but also in an eminent degree with the elements of a bacillus-filament or leptothrix. In each case, indeed, it is possible to ascertain that all lengths occur from the cubical or spherical element to the cylinder or rod. The former elongate into the latter and then divide. According to whether the division occurs in a short or long element, the daughter elements are cubical or spherical in the former, cylindrical or rod-shaped in the

FIG. 40.—BACILLUS SUBTILIS GROWN IN PORK BROTH.
At 1, the elements are thickened. The preparation had been dried and stained with aniline purple.

latter case. This applies to single bacilli, to short chains, and to the leptothrix forms.

There are a great many species of bacilli, differing from one another (*a*) in the shape of the elements, (*b*) in motility, (*c*) in the power of forming filaments or leptothrix, and particularly (*d*) in the thickness and length of the elements.

(*a*) There are some species of bacilli—*e.g.* hay-bacillus, anthrax-bacillus, bacillus of putrid blood, bacillus found

occasionally in the blood-vessels of dead animals, bacillus of malignant œdema (Koch), &c.—in which in the single bacilli and in the chains and filaments, the size of the elements varies from that of a cubical or spherical mass of protoplasm to that of a cylinder or rod several times as long as it is thick. In some species (*e.g.* tubercle-bacilli), the elements are almost spherical. There are on the other hand other species (*e.g.* bacillus amylobacter) where the elements are always rods or cylinders. In these cases of short bacilli it sometimes becomes difficult to say whether one has to deal with bacilli or bacteria, but the growth of the bacilli into leptothrix, and particularly their power of forming spores, is decisive, although neither of these events may happen owing to peculiar conditions.

(*b*) Some bacilli (*e.g.* hay-bacillus, bacillus in common putrefaction, bacillus growing on surfaces of putrefying material and tissues, bacillus found in the abdominal organs after putrefaction has set in, &c.), are possessed of a flagellum at one end, and are therefore endowed with the power of locomotion. Other species (*e.g.* anthrax-bacillus, bacillus of malignant œdema) are without such power. But even in the first case the power of locomotion is possessed by the bacilli only when single or in short chains, not by the longer chains or leptothrix.

(*c*) Not all bacilli are capable of forming leptothrix-filaments. This power is possessed in an eminent degree by certain species, such as the hay-bacillus, the anthrax-bacillus, the bacillus of malignant œdema, the bacillus found on the surface of the mucous membrane lining the cavity of the mouth and tongue (leptothrix buccalis). Other bacilli (*e.g.* bacillus amylobacter, leprosy-bacillus, tubercle-bacillus, &c.), generally do not form leptothrix.

(*d*) There exists the greatest variety in reference to the

thickness of the bacilli; some (*e.g.* bacillus amylobacter, and some species occurring in ordinary putrefaction) being several times as thick as others, like hay-bacillus, anthrax-bacillus, &c.

Many bacilli and bacillus-filaments (*e.g.* hay-bacillus, anthrax-bacillus) degenerate on growing old, the protoplasmic elements becoming granular and breaking down altogether into debris. This may occur to single elements within a chain or leptothrix; and then the corresponding part of the sheath of the chain, owing to the subsequent disappearance of the debris, becomes empty and devoid of protoplasm. Longer or shorter portions of a chain or leptothrix may thus degenerate and become deprived of protoplasm, the sheath only persisting. These portions become at the same time thicker, the sheath having swollen up.

Another mode of degeneration consists in the elements and sheath curling up, swelling up, and ultimately breaking down into debris. According to Cohn,[1] bacilli do not form zooglœa in the same way as micrococcus and bacterium do. With all due deference to the authority of Cohn, I must hold that the bacilli possessed of a flagellum are capable of forming a true zooglœa. When one inoculates a fluid-nourishing medium (*e.g.* broth) with hay-bacillus or other motile bacillus of common putrefaction, after keeping it for twenty-four hours in the incubator one notices a uniform turbidity. After several days one notices that the surface of the fluid becomes covered with a whitish film; this, as incubation goes on, thickens into a thick resistant not very friable pellicle. By shaking the fluid the pellicle becomes detached from the glass wall and sinks to the bottom of the fluid; after another day or two a new pellicle is formed, and so on until the material is exhausted.

[1] *Beitr. z. Biologie d. Pflanzen*, vol. ii.

Any part of this pellicle examined under the microscope shows itself to be a zooglœa in the true sense of the word, vast numbers of shorter or longer bacilli crossing and interlacing and lying embedded in a gelatinous hyaline matrix. As with bacterium termo, one occasionally notices at the margin of the mass one or other bacillus wriggling itself free and darting away. And in the case of non-motile bacilli, putrefactive and others, I have also seen distinct formations of zooglœa, having the shape of spherical or oval lumps of various sizes composed of a hyaline jelly-like matrix, in which are embedded the bacilli in active multiplication.

In those species in which the bacilli are capable of forming leptothrix (leptothrix buccalis, hay-bacillus, anthrax-bacillus) the filaments may form dense convolutions. When in these convoluted filaments spores are formed (see below) and the sheaths of the filaments swell up and become agglutinated into a hyaline jelly-like substance, the spores appear to form a sort of zooglœa.

Bacilli are killed by drying, but it is necessary to bear in mind that they must be exposed to the drying process in thin layers (Koch). At the temperature of boiling water they are invariably killed, but not their spores. Even heating them from half an hour to several hours at a temperature above 55° or 60° C. kills them. Freezing also kills them, but not their spores. Carbolic acid, corrosive sublimate, thymol, &c., kill them.

One of the most striking phenomena in the growth of bacilli is their power of forming spores. These are generally oval when fully developed, spherical when immature; they are of a glistening appearance, and take the ordinary dyes either with difficulty or not at all; they are generally a little thicker than the bacilli within which they have developed. Their formation always takes place in this way: in one or

other elementary cubical, spherical, or rod-like mass of protoplasm there appears a bright dot; this enlarges at the expense of the protoplasm until in its fully developed state it has an oval shape. The whole of the protoplasm of an

FIG. 41.—THE SAME BACILLUS AS IN PRECEDING FIGURE.
At 1, spores have made their appearance.

element is not consumed in this process, a small trace always remaining unused at one or both ends. The sheath enlarges and the bacillus looks much thickened; then the sheath breaks, and the spore with the remnant of protoplasm

FIG. 42.—THE SAME BACILLUS AS IN PRECEDING FIGURE.
Some of the spores are germinating into bacilli.

FIG. 43.—BACILLUS SUBTILIS OF HAY INFUSION.
At 1, spores are germinating into bacilli.

becomes free. Soon this remnant disappears, if it had not disappeared while the spore was still contained within the sheath, and now the spore is free. Under the most favourable conditions a spore may be formed in each

elementary mass of protoplasm, or it may be only in a small number. In the first case: a consecutive series of spores is present in the bacilli, two spores if the bacillus is composed of two elementary cells, four in a chain of four elementary cells, or a vast number in a leptothrix. In the second case: a bacillus composed of two or four elementary cells may contain only one spore at one end or in the middle, or one at each end, or two together in the middle; in the leptothrix spores are seen only at comparatively long intervals. The position of the spore in the bacillus is generally so that the long axis of the spore is parallel to that of the bacillus; but exceptionally it may be placed obliquely or even transversely. The bacilli in which spore-formation has set in are always much thicker, twice or more, than those in which no spore-formation has occurred; and as has been stated above, the sheath swells up and remains for some time as a hyaline gelatinous capsule around the spore, but sooner or later this is also lost and the spore becomes quite free. When spore-formation has taken place in a convolution or in a mass of leptothrix, and after the sheaths of the bacilli have become swollen up into a gelatinous matrix, it looks as if we had a zooglœa, in which the bright oval spores form the particular elements embedded in a more or less hyaline gelatinous matrix. But even in these cases on careful analysis it is noticed that the spores have a linear or serial arrangement, being originally developed in filaments.

This spore-formation occurs in many species of bacilli, and it closes the cycle of the life-history of the bacilli. But it does not take place under all circumstances. In the case of many bacilli, *e.g.* hay-bacillus, anthrax-bacillus, bacillus of putrefaction, spore-formation occurs only when there is an ample supply of oxygen, *e.g.* when the bacilli grow on the surface of the nourishing material (Cohn, Koch). It has

nothing whatever to do with the exhaustion of the nourishing material, as Buchner seems to think; for if the conditions of spore-formation are given, amongst these particularly the exposure to the air, bacilli will commence to form spores long before the nourishing material is exhausted. I will here mention a particular instance to show this.

Take a test-tube with Agar-Agar peptone, such as has been mentioned in a former chapter as fit for inoculation; inoculate the surface of the Agar-Agar with hay-bacillus or anthrax-bacillus, place it in the incubator, and keep it there at a temperature of 30° to 35° C. After 36 to 48 hours you will find the surface covered with a good crop of bacilli and leptothrix, and in some of them spore-formation is already going on with great vigour. For several days after, the amount of leptothrix increases, and in the filaments large numbers of spores are formed. This goes on for several weeks, long before the nourishing material becomes exhausted. But during all this time the spore-formation is limited only to the surface; the filaments growing into the deeper strata remain without spore-formation. The same observation can be made with gelatine mixtures of peptone, broth, &c., in the test-tube or in the glass cell described and figured in Chapter V. In the case of the gelatine mixture it is particularly instructive to watch this process, since it clearly proves that the free access of air is essential for the formation of spores. For if the anthrax-bacillus be grown on the (solid) mixture of gelatine and broth described in a former chapter and kept at the ordinary temperature of the room or in the incubator at not more than 22° to 25° C., the spore-formation on the surface occurs only as long as the material remains solid. Anthrax-bacillus as it grows liquefies the gelatine mixture; in consequence of this after some days the superficial layers of the material become fluid, and the bacillar growth, sinking to

the bottom of the fluid layer, is thus removed from the surface. The spores which were freely formed while the growth went on on the surface, germinate again into bacilli, but because these have now sunk into the depth, although rapidly multiplying and growing into filaments, they cease to form any spores.[1]

Bacilli which are not possessed of the power of locomotion (*i.e.* are without a flagellum) when sown into the depth of a fluid or solid material, if they have no chance, accidental or otherwise, of reaching the surface, do not as a rule form spores; but there are some such bacilli which, although not growing on a free surface, nevertheless form spores, *e.g.* the bacillus butyricus or amylobacter (Prazmowski). Some putrefactive bacilli occurring after death in the abdominal organs (intestine, kidney, spleen, liver), and in fluid exudations within the peritoneal and pleural cavities, show also spore-formation; probably they get their oxygen from the tissues. Anthrax-bacillus, however, never forms spores except it is growing well exposed to the outer air.

The bacilli which are possessed of a flagellum (*e.g.* hay-bacillus, bacillus of putrefaction) generally form a pellicle on the surface, and in this pellicle copious spore-formation goes on.

The spores first formed, when shaken down into the fluid, again germinate into bacilli, and there multiply. The last pellicle formed in a culture, before exhaustion, represents the last crop of spores; and these, owing to the exhaustion of the nourishing fluid, remain as spores, only capable of germinating into bacilli when new nourishing material is added, or when they are transplanted to new nourishing material.

[1] *Report of the Medical Officer of the Local Government Board*, 1881.

It is a rule that wherever the spores are formed they germinate into bacilli if they have access to nourishing material; but if not, or if the nourishing material is exhausted, they remain as spores. Spore-formation does not take place at low temperatures. Koch found in the case of anthrax-bacillus that a temperature below 12° C. prevents the formation of spores. Pasteur states that in the case of anthrax-bacillus spore-formation does not take place above 40° C.; never for instance at 42° or 43° C. Koch gives 43° as the upper limit; but I have found that both in the case of hay-bacillus and anthrax-bacillus the bacilli form spores copiously even at a temperature of 44° C. Moisture is an essential element in the formation of spores.

The spores represent the seeds capable of retaining life and of germinating into bacilli even after what would appear the most damaging influences (that is, damaging to all other kinds of organisms and to the bacilli themselves), such as long lapse of time, drying, heat, cold, chemical re-agents, &c. Spores retain the power to germinate into bacilli after the lapse of long periods, and there is no reason to assume that these periods have any limit; it makes no difference whether they are kept dry or in the mother-liquid.

The temperature of boiling water, while it kills micrococci, bacteria, and bacilli themselves, does not affect the vitality of the spores. Cohn (*loc. cit.*) found spores of hay-bacillus still capable of germination even after boiling; boiling for half an hour or more killed them. Prazmowski found that the spores of bacillus butyricus (amylobacter) are killed by five minutes' boiling. In the case of bacillus subtilis, *e.g.* hay-bacillus, I found that boiling for half an hour does invariably kill them, but ten minutes is not to be relied on. Exposing the spores of anthrax-bacillus to a temperature of 0° to − 15° C. for one hour did not kill them. Antiseptics, such as carbolic acid

(5 to 10 per cent.), strong solutions of phenyl-propionic acid and phenyl-acetic acid, corrosive sublimate (1 : 10,000), although the spores were kept in these fluids for twenty-four hours and more, did not kill them.

The spores of bacillu-santhracis are killed by one to two minutes' boiling in water or salt-solution.

This great resistance of spores to low and high temperatures, to acids and other substances, is due to this, that the substance of each spore is enveloped in a double sheath : an internal sheath probably of a fatty nature, and an external one probably of cellulose ; both are very bad conductors of heat.

Owing to the fact that spores resist the action of boiling water, if not prolonged for ten minutes, and that the other bacteria (such as micrococcus, bacterium, and bacillus itself) are killed by the temperature of boiling water if kept at this temperature for a few seconds, it is possible to separate the spores of bacilli from the other organisms. All one has to do is to subject the fluid containing these various organisms to the temperature of boiling water for a few seconds. All except the spores of bacilli will be thereby killed, and thus the fluid becomes free of all other organisms except the spores.

When spores are sown in a nourishing material, fluid or solid, and when this is exposed to a temperature of about 32° to 38° C., the spores after the lapse of a few hours, in some cases six (spores of anthrax-bacillus), in others two to four hours (spores of hay-bacillus), in others more than six hours, are seen to germinate, each spore growing into a bacillus. In the case of solid nourishing material the presence of moisture is essential.

In this germination what one sees is this: the spore increases in thickness, it then loses its dark contour at one

pole or at one of the long sides, and at this point a pale projection appears. This projection increases in length and gradually becomes as long as a bacillus, the investment of the spore gradually fading away. This new bacillus soon divides into two, and so on.

The spores are capable of germinating independently of the free access of air.

It has been shown by Engelmann that the presence and renewal of oxygen as well as a certain concentration of the nutritive material is essential for the motility of those bacteria that are possessed of cilia, *i.e.* that are possessed of locomotion.

As long as the bacteria are living, their protoplasm does not combine (stain) with nitrate of silver solution, only after death does this become possible. Hereby an index is furnished for ascertaining whether, and which, bacteria in a given sample are living, and which are dead. There is no difference in this respect, *i.e.* in respect of the different reaction of nitrate of silver on living and dead protoplasm, between the protoplasm of bacteria and that of other vegetable or animal tissues.

All bacteria, pathogenic and non-pathogenic, require for their growth and multiplication oxygen, which they either obtain from the medium in which they grow, and which oxygen is dissolved in those media, or if this is consumed or absent it is obtained by the bacteria in the process of the chemical decomposition of the carbohydrates and proteids present. Dr. Dupré[1] has shown that the presence or disappearance of oxygen (air) dissolved in water is a precise gauge, in the first case of the absence, in the second of the growth, of microphytes.

[1] *Report of the Medical Officer of the Local Government Board,* 1884.

CHAPTER X.

BACILLUS: NON-PATHOGENIC FORMS.

SEPTIC bacilli.

(*a*) *Bacillus subtilis* (hay-bacillus).—The elementary rods are of various lengths from 0·002 to 0·006 mm., and are

FIG. 44.—FROM A CULTURE OF BACILLUS SUBTILIS (HAY-BACILLUS).
Various forms between single bacilli and leptothrix.
Magnifying power about 700.

about 0·002 mm. in thickness. According to Cohn[1] at a temperature of 21° C. division into two requires

[1] *Loc. cit.*

about one hour and a quarter, at 35° C. only about twenty minutes.

The bacilli are capable of forming leptothrix filaments. The bacilli when single are possessed of one flagellum, or sometimes of two, one at each end. After division the individual bacilli remain connected, each possessing a flagellum at the free end. Each of them divides again into four, so that a chain of four is formed. But they may separate again or may go on dividing, remaining united, and thus forming a longer or shorter filament. Not all

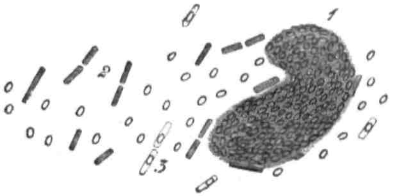

FIG. 45.—FROM A CULTURE OF BACILLUS SUBTILIS (HAY-BACILLUS), WITH COPIOUS FORMATION OF SPORES.

1. Mass of spores embedded in hyaline matrix.
2. Bacilli.
3. Single bacilli containing each a spore: the sheath of the bacilli is well seen.

Magnifying power about 700.

bacilli possess the flagellum, many of them being for a time in a resting state.

The bacilli form a dense resistant pellicle on the surface of the nourishing medium, and in this copious spore-formation takes place. If shaken when growing in a fluid, the pellicle falls to the bottom, and soon a new pellicle is formed.

Spore-formation is independent of any deficiency of nourishing material. The spores are oval, bright, of about 0·001 to 0·002 mm. in length, and about 0·0006 to 0·001 mm. in

thickness. They do not stain in ordinary dyes, and hence form a great contrast to the bacilli.

This bacillus is very common and widely distributed; it occurs in almost every organic substance rich in nitrogenous compounds which is left exposed to the air to decompose. The best material is hay-infusion. An infusion, cold or hot, of hay is made in a beaker or flask; the fluid is filtered, covered with a glass plate, and left to stand in a warm place. After a day or two it swarms with bacillus subtilis, which is also called hay-bacillus, since ordinary hay contains multitudes of its spores. For this reason even boiling of the fresh infusion for a few minutes does not sterilise it.

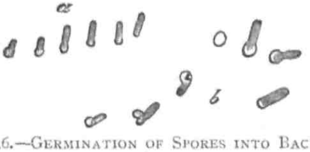

FIG. 46.—GERMINATION OF SPORES INTO BACILLI.

a. Spores of a small kind.
b. Spores of a larger kind of bacillus subtilis.
Magnifying power about 700.

The bacillus grows well in every fluid that contains the necessary salts and nitrogenous compounds; thus all kinds of broth, all kinds of animal fluids (hydrocele, blood-serum, &c.), gelatine, peptone solution, &c., are suitable nourishing media.

The spores of the hay-bacillus are widely distributed in the air, and most contaminations by air are due to its spores.

(*b*) *Bacillus ulna.*—By this name Cohn[1] designates certain species of bacilli, stiffer and thicker than those of bacillus subtilis. The individual elements are about 0·01 mm. long,

and 0·002 mm. thick. They are motile, just like bacillus subtilis. Although they form chains they do not form proper leptothrix. They occur in putrid fluid. They are very common in the ichor produced by injecting ammonia or other substances producing sloughing and necrosis of the subcutaneous tissue in the guinea-pig.

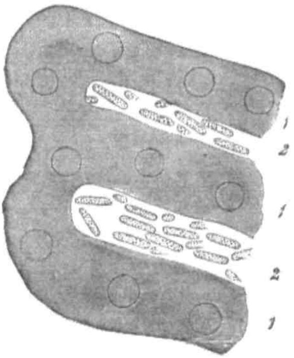

FIG. 47.—BACILLUS ULNA, IN THE CAPILLARIES OF THE HUMAN LIVER. POST-MORTEM CHANGE.

1. Liver cells, somewhat swollen.
2. Bacilli.

Magnifying power 300.

(*c*) *Bacillus septicus* occurs in earth, in putrid blood, and in many putrid albuminous fluids. It is non-motile, and is capable of forming leptothrix. The thickness varies from 0·004 to 0·01 mm., and its length depends on the number of elements contained in a row. The shortest are about 0·004 mm. There are various species, differing from one another in the thickness of the elements. They are all anaërobic. The elements, whether in the short rods or in the leptothrix filaments, are cubical or rounded. The rods and filaments are markedly rounded on the ends. It forms spores

independently of free access of air. The spores are oval, and differ in thickness according to the thickness of the bacilli they are formed in. The bacillus is found occasionally in the blood-vessels of man and animals after death. In a nourishing fluid, in which micrococcus, bacterium termo, or bacillus subtilis grows, they have no chance of growing, and even when numerous at first they soon disappear.

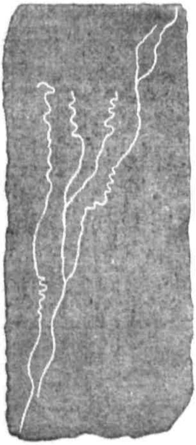

FIG. 48.—STREPTOTHRIX FOERSTERI (AFTER COHN).

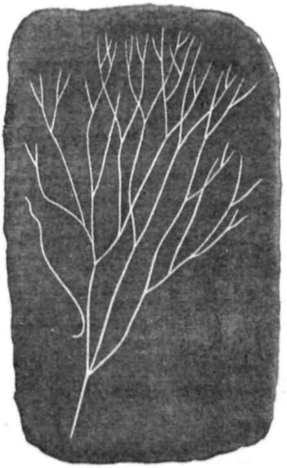

FIG. 49.—CLADOTHRIX DICHOTOMA (AFTER COHN).

(*d*) *Streptothrix* and *Cladothrix*.—Cohn[1] found in a concretion of the human lacrimal canals long, pale, smooth, apparently branched threads, either straight or twisted; they were finer than the threads of leptothrix buccalis; he called them *Streptothrix Foersteri*. They are probably identical morphologically with *Cladothrix dichotoma*. This latter

[1] *Beitr. z. Biol. d. Pflanzen*, vol. i. p. 186.

occurs in pond-water containing decomposing organic matter. It consists of long whitish threads fixed on chlorophyll-containing algæ. The threads when fresh appear smooth, pale, occasionally granular, and on staining they are seen to be composed of shorter or longer bacilli just like the leptothrix form of bacillus subtilis; but they are thicker than the bacillus subtilis. Occasionally the ends of the threads are seen not as linear series of bacillar rods, but like bacillus anthracis and the bacillus of blue milk (see below) as chains of torula-like spherical elements. From the threads single motile bacilli are seen to come off. The threads are only apparently branched, since the branches are threads merely stuck on to other threads sideways at an acute angle. A bacillus may be seen to stick to a thread and then to grow out by continuous divisions into a long chain of bacilli, thus forming, as it were, a side-branch. Some of the threads are wavy and curved; most of them are, however, straight. Zopf[1] claims to have observed that the threads of the cladothrix gave rise to micrococcus, bacterium, bacillus, and spirillum; and states that each of these is again capable of growing into the threads of the cladothrix. But these observations were not made after exact methods.

(*e*) *Beggiatoa.*—In stagnant water, particularly in sulphur-containing water, peculiar oscillating colourless threads are met with of the thickness of 0·0001 to 0·016 mm.; they contain highly refractive granules, which Cohn (*Beiträge zur Biol. d. Pfl.* i. 3) has shown to be composed of sulphur. After dissolving these granules it is seen that each thread is septate, being composed of a sheath and transverse septa at regular intervals, by which the threads appear made up of a

[1] *Zur Morphologie der Spaltpflanzen*, Leipzig, 1882; see also Cienkowski.

Caucascus, and which is used as ferment to produce from cow's milk a peculiar drink called "kephir" or "hyppö." The bacillus is similar to the bacillus subtilis, but is distinguished from it and all other bacilli by this, that every bacillus forms two spores, one at each end, hence the name dispora. But after recent investigations it appears that this bacillus is accidental, the fermentation being produced by saccharomyces mycoderma (see Chapter XIV.)

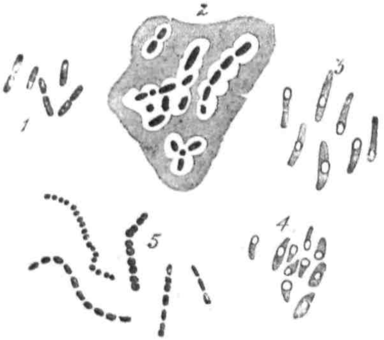

FIG. 52.—BACILLUS SYNCYANUS (AFTER NEELSEN).

1. Typical bacilli, motile.
2. Non-motile rods invested in a gelatinous capsule.
3 and 4. Bacilli in which spore-formation is going on.
5. Torula-form of the bacillus.

Pigment bacilli.

(*a*) *Bacillus ruber.*[1]—This appears as minute rods, isolated or in twos and fours, and motile. It was found on boiled rice by Frank. Its colour is red, and contained in the bacilli themselves.

(*b*) *Bacillus erythrosporus.*—Motile isolated rods and leptothrix. It was found in meat-extract solutions and on

[1] Cohn. Frank. *Beitr. z. Biol. d. Pflanzen*, vol. iii. p. 181

decomposing albumen, and forms pellicles. In the rods are found oval spores.[1]

(*c*) *Bacillus syncyanus* (Neelsen) causes the blue colour of milk after the milk has become acid; it grows well in ammonium lactate. It consists of motile rods, single or in short chains. Neelsen[2] saw the bacilli assuming a torula-form, the individual cells being hourglass-shaped or oval, or even spherical (compare *Bacillus anthracis*, Chapter XI.). The rods appear also as a non-motile variety, and are then found to be invested with a thick hyaline gelatinous envelope. The rods form bright oval spores, being at the same time swollen up and ovoid. In Cohn's nourishing fluid they are capable of forming leptothrix, in which occur here and there huge spherical or oval swellings, which, according to Neelsen, probably represent gonidia.

[1] Cohn and Miflet, *Beitr. z. Biol. d. Pflanzen.* vol. iii. p. 128.
[2] *Beitr. z. Biol. d. Pflanzen*, vol. iii. p. 187.

bacilli. Koch cultivated these bacilli artificially on mixtures of aqueous humour and gelatine, of gelatine and peptone (1 per cent.), salt (0·6 per cent. NaCl), and sodium phosphate in sufficient quantity to just produce an alkaline reaction. The bacilli grow well on this mixture, and by repeated and rapid division form peculiar branched series.

FIG. 55.—FROM A SECTION THROUGH A LYMPHATIC GLAND OF MAN DEAD OF SEPTICÆMIA.

1. A blood-vessel which at one place is distended by and filled with minute bacilli.
2. Lymph-corpuscles.
3. Degenerated lymph-corpuscles.

Magnifying power 700. (Stained with gentian violet.)

(*b*) *Bacillus of septicæmia of man.*—In several cases of human septicæmia I have found in the blood-vessels of the swollen lymphatic glands large numbers of minute bacilli,

slightly thicker than those just mentioned. They form continuous masses, both in the capillaries and in the minute veins, amounting in some cases to veritable emboli. They occur isolated or in short chains, their length about 0·001 to 0·0025 mm., their thickness about 0·0003 to 0·0005 mm. Arloing and Chauveau (mentioned in the *British Medical Journal*, Jan. 12, 1884) found in gangrenous septicæmia around wounds short bacilli, some containing one or two spores, which they consider as the true cause of the gangrene. They are destroyed when fresh by a temperature varying between 90° and 100° C.; after drying, a temperature of 120° C. is required.

FIG. 56.—FROM A SECTION THROUGH THE MESENTERIC GLAND OF A PERSON WHO DIED OF TYPHOID FEVER.

1. Capillary blood-vessel filled with blood-corpuscles.
2. Large lymph-cell.
3. Nuclei.
4. Bacilli.

Magnifying power 700.

(*c*) *Bacillus of typhoid fever of man.*—Klebs[1] described in the inflamed Peyer's glands, in the mesenteric glands, larynx, and lungs of patients dead of typhoid fever, certain bacilli, which are about 0·0002 mm. thick and of various lengths, forming filaments up to 0·05 mm. long. These bacilli form

[1] *Archiv f. exp. Path.* vol. xii.

spores. Eberth[1] found in about 50 per cent. of cases of patients dead of typhoid fever, in the mesenteric glands and spleen, peculiar short bacilli, rounded at their ends, and occasionally slightly constricted in the middle; some of them contained spores. The bacilli stain very freely with methyl-violet. It is, however, doubtful whether these bacilli can be considered as necessarily and intimately connected with typhoid fever, seeing that they are not constant, and only occur in the mesenteric glands and spleen, *i.e.* in localities into which an immigration of putrefactive bacilli from the bowels may easily take place; especially when we remember that in fatal cases of typhoid fever there constantly occur severe sloughing and necrosis of the Peyer's glands. (See end of Chapter XVIII.) The bowels in typhoid fever always contain innumerable masses of micrococci in colonies; and these micrococci are found not only in the tissue of the intestinal mucous membrane but also in the mesenteric glands and spleen.[2]

(*d*) *Bacillus of choleraic diarrhœa from meat-poisoning.*— In July, 1880,[3] there occurred in Welbeck, Notts, an extensive outbreak of diarrhœa among over seventy-two persons who had partaken of beef and ham sandwiches sold at Welbeck on the occasion of a sale of timber and machinery on the estate of the Duke of Portland. The infection showed itself after an incubation-period varying from twelve hours or less to forty-eight hours or more. The first symptoms were a sudden feeling of languor, nausea, griping in the abdomen, in some cases giddiness and fainting, and

[1] *Virchow's Archiv*, vols. lxxxiii., lxxxvii. See also Koch, *Mittheil. a. d. k. Gesundheitsamte*, i. 1881; and Gaffky, *ibid.* 1882.

[2] Klein, *Reports of the Medical Officer of the Privy Council*, 1875.

[3] Report by Dr. Ballard in the *Reports of the Medical Officer of the Local Government Board*, 1880.

pain in the trunk. Then followed pain in the abdomen, diarrhœa, and vomiting, the diarrhœa being most constant. Four cases ended fatally. On *post-mortem* examination

FIG. 57.—FROM A SECTION THROUGH THE KIDNEY OF A CASE THAT DIED AFTER MEAT-POISONING AT WELBECK.

The figure represents part of a glomerulus of a Malpighian corpuscle, in which some of the capillary blood-vessels are filled with the bacilli. Magnifying power 700.

1. Capsule of Malpighian corpuscle.
2. Capillaries filled with bacilli.
3. Capillaries empty.
4. Bacilli contained between capillaries.

enteritis and pneumonia were most prominent. Part of the kidney was examined in microscopic sections, and it was found that many of the tubuli uriniferi contained hyaline

spores. Eberth[1] found in about 50 per cent. of cases of patients dead of typhoid fever, in the mesenteric glands and spleen, peculiar short bacilli, rounded at their ends, and occasionally slightly constricted in the middle ; some of them contained spores. The bacilli stain very freely with methyl-violet. It is, however, doubtful whether these bacilli can be considered as necessarily and intimately connected with typhoid fever, seeing that they are not constant, and only occur in the mesenteric glands and spleen, *i.e.* in localities into which an immigration of putrefactive bacilli from the bowels may easily take place ; especially when we remember that in fatal cases of typhoid fever there constantly occur severe sloughing and necrosis of the Peyer's glands. (See end of Chapter XVIII.) The bowels in typhoid fever always contain innumerable masses of micrococci in colonies ; and these micrococci are found not only in the tissue of the intestinal mucous membrane but also in the mesenteric glands and spleen.[2]

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casts; that the capillaries of the glomeruli of the Malpighian corpuscles, and the afferent arterioles, contained numbers of bacilli, some of the capillaries being distended by and plugged with masses of bacilli densely aggregated. In February, 1881, a similar but less extensive outbreak occurred at Nottingham, among fifteen persons that had partaken of certain baked pork. The symptoms were similar to those in the Welbeck outbreak. One case ended fatally. *Post-mortem:* bloody exudation in pericardium, intense pneumonia, mesenteric glands enlarged, enteritis, Peyer's glands enlarged. Bacilli similar to those of the above case were found in the blood, in the pericardial exudation, in the juice and in the bloody fluid filling the

FIG. 58.—ISOLATED BACILLI IN A SMALL ARTERY OF THE SAME KIDNEY AS IN PRECEDING FIGURE.

Some bacilli contain spores.

alveolar cavities of the inflamed lung, in the vessels of the kidney, in the submucosa of the inflamed Peyer's glands of the small intestine, in the blood-vessels of the spleen and around them.

The bacilli vary in length between 0·003 and 0·009 mm.; their thickness is about 0·0013 mm. They are rounded at their extremities, single or in chains of two, and some contain a bright oval spore, situated in the centre or at one end, and about 0·001 mm. thick. This was the case with the bacilli in the glomeruli of the kidney of the Welbeck case. The bacilli containing spores were thicker than those without them.

Experiments by feeding and inoculation made on dogs and cats, rabbits, guinea-pigs, and mice, with the ham that had done the mischief in the Welbeck case produced positive results. In all cases we found pneumonia and hæmorrhage in the liver, peritonitis in some, spleen enlarged in most. The bacilli found in this ham were cultivated in the incubator in white of egg, and after two days' cultivation four white rats, and several guinea-pigs and white mice were inoculated, and they became ill after twenty-four hours; they were quiet, did not feed well, and were more or less soporous. When killed the spleen was found enlarged, and in the lungs were found hæmorrhage and hyperæmia, and in some cases extensive pneumonia.

Blood, pericardial exudation, and lung juice from the fatal Nottingham case inoculated into ten animals (guinea-pigs and white mice) produced fatal results in six, the other four were killed: but in all there was severe pneumonia, in eight out of the ten there was peritonitis, in four also pleuritis, and in two in addition enlargement of the liver and spleen. Bacilli were found in the blood and exudations of these animals. On cultivating blood and lung juice from the above case a crop of bacilli was produced, which on inoculation proved very poisonous in the same way as in the previous cases.[1]

(*e*) *Bacillus malariæ.*—Klebs and Tommasi-Crudeli[2] described a bacillus occurring in the soil of the Roman Campagna, which they cultivated on gelatine. The rods are about 0·002 to 0·007 mm. long; they grow in cultures into long leptothrix filaments composed of short joints. The rods form spores either in the centre or at their ends. They grow well also in other media, *e.g.* albumen, urine, and glue.

[1] Compare also Huber, *Archiv f. klin. Med.* xxv.
[2] *Archiv f. exp. Path.* vol. xi.

Quite recently Marchiafava and Celli[1] found that the red blood discs of patients affected with recent malaria contain peculiar homogeneous bodies, possessed of amœboid movement, in size a fraction of that of the blood discs. They call them *hæmoplasmodium malariæ.* Sometimes these plasmodia include pigment granules assimilated from the pigment of the blood discs. Such pigmented plasmodia had been already noticed by Laveran. Marchiafava and Celli found that malaria blood containing the plasmodia is capable of producing intermittent fever in man after intravenous injection. The blood corpuscles of a person so infected again contain the hæmoplasmodia.

(*f*) *Bacillus of ulcerative stomatitis in the calf.*—In the *Lancet* of May, 1883, A. Lingard and E. Batt described peculiar bacilli in ulcerations occurring on the tongue and buccal mucous membrane of the calf. "The typical ulcer in advanced cases consists of a sore with free overhanging edges. On section through the sore the tongue is found necrosed to a considerable depth." "Whenever the sore touches any other part of the mouth or cheek, the disease is communicated and rapidly spreads. In some cases similar necrotic changes had taken place in the lung. The line of junction of the necrotic with the healthy tissues was found to be occupied by a dense mass of bacilli having the appearance of a dense phalanx advancing upon the healthy tissues. The disease has been proved capable of transmission (to the rabbit and mouse) by injection of the bacilli in question, which are equally numerous and virulent after passing through several generations by inoculation."

The disease often ends fatally in calves.

The best method of staining the bacilli was found to be

[1] *Fortschritte d. Med.* Nos. xi., xviii., xxiv., 1885.

this: The sections, both those prepared from the ulcerations of the calf's tongue and from the inoculated tissues of the rabbit, are immersed in a mixture of magenta and methyl blue, then washed in spirit, and after clarifying in clove-oil are mounted in Canada-balsam solution. The bacilli are

FIG. 59—FROM A SECTION THROUGH NECROSED AND ADJOINING INFLAMED PARTS OF THE EAR OF A RABBIT, INOCULATED WITH MATTER TAKEN FROM ULCERATIVE STOMATITIS OF THE CALF.

1. Necrosed part.
2. Inflamed tissue.
3. Bundles of bacilli.

Magnifying power 700. (Stained with magenta.)

stained deep pink, the inflamed tissue blue. The bacilli appear as thin rods in rows, thus forming a leptothrix-like growth. In some of the long filaments the individual bacilli are not well shown. The filaments are either straight or more or less curved. The length of the single bacilli varies

from 0·004 mm. or less to 0·008 mm. or more; the thickness is about 0·001 mm. Many of them contain spores. In the ear of the rabbit they invade the connective tissue as well as the cartilage over the whole extent of the ulceration and its

FIG. 60.—FROM A SECTION THROUGH TONGUE OF CALF, ULCERATIVE STOMATITIS.

1. Muscular fibres.
2. Inflamed tissue.
3. Bundles of the bacilli.

Magnifying power 700. (Stained with magenta.)

neighbourhood. Mr. Lingard found the same bacilli, having the same arrangement, in a case of noma in the human subject.

(*g*) *Bacillus of glanders.*—In 1882 Schütz and Löffler[1] ascertained the occurrence of peculiar bacilli in the nodules

[1] *Deu'sche med. Wochenschrift*, 52, 1882.

of the nasal mucous membrane and internal organs, such as the lung, spleen, and liver, of horses dead or dying from glanders. These bacilli are very minute, being of about the size of tubercle-bacilli (see below), and are brought out by staining the tissues with a concentrated watery solution of methylene-blue, and after this washing with very dilute acetic

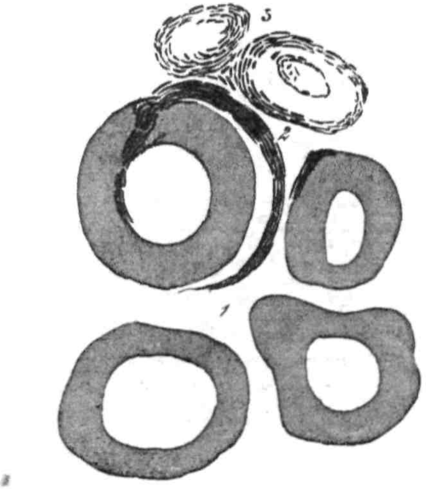

FIG. 61.—FROM A SECTION THROUGH THE CARTILAGE OF RABBIT'S EAR IN WHICH ULCERATION HAD BEEN PRODUCED BY INOCULATION WITH NECROSED MATTER OF CALF'S TONGUE.

1. Cartilage capsules.
2. Bundles of good bacilli.
3. Bundles of degenerating bacilli.

Magnifying power 700. (Stained with magenta.)

acid. They succeeded in artificially cultivating the bacilli in the incubator at 38° C., on solid sterilised serum of horses' and sheep's blood, using for the purpose particles (see below under "Tubercle-bacilli") of nodules of the lung and spleen of a horse dead of glanders. After two days (*i.e.* on the

third day) there appeared on the surface of the inoculated material the first traces of the growth in the form of minute transparent droplets which consisted entirely of the characteristic bacilli. Cultivating these through several generations or transferences, and then inoculating with them a horse, rabbits, guinea-pigs, and mice, positive results were obtained, especially in the guinea-pigs, which appear very susceptible to the disease. On the site of the subcutaneous inoculation appears an ulcer with indurated base, speedily enlarging and spreading; other ulcers follow in the

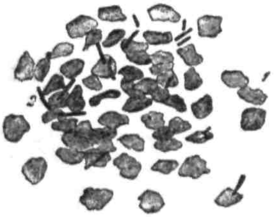

FIG. 62.—PUS OF A PULMONARY ABSCESS IN A HORSE DEAD OF GLANDERS.

1. The nuclei of pus cells.
2. The glanders-bacilli.

Magnifying power 700. (The preparation had been stained with methylene-blue.)

neighbourhood, the neighbouring lymphatic glands become swollen, and the general infection follows, in the form of nodules and ulcers on the nasal septum, and nodules in the internal organs. In the guinea-pig a characteristic tumour of the testis, ovary, and vulva is often observed. In all these cases the diseased tissues and organs contained the characteristic bacilli.[1]

[1] Other writers on the bacilli of glanders are Drs. Bouchard, Capitan, Charvin, in the *Revue médicale française*, Dec. 30, 1882. N. P. Wassilieff, in *Deutsche med. Woch.* 11, 1883, observed the bacilli in human glanders.

(*h*) *Bacillus of swine plague.*—In a report to the medical officer of the Local Government Board for 1877-1878, I have shown that in this acute infectious disease the lungs, intestines, and serous membranes are commonly found affected. The lungs show lobular and lobar pneumonia, the large intestine contains hæmorrhages and ulcers, while the serous membranes and lymph-glands are inflamed. Occasionally, but not often, also the skin shows irregular red patches. I have shown that in this disease the diseased organs contain a form of bacillus, which only by its small size differs from

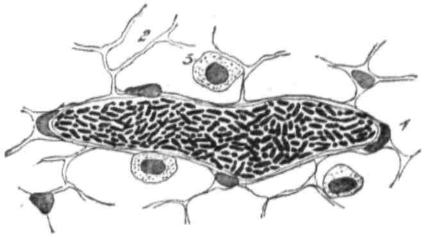

FIG. 63.—FROM A SECTION THROUGH THE INFLAMED INGUINAL LYMPH-GLAND OF A PIG DEAD OF SWINE PLAGUE.

1. A capillary blood-vessel filled with bacilli.
2. Reticulum of adenoid tissue.
3. A lymph-cell.

Magnifying power 700. (Stained with Spiller's purple.)

bacillus subtilis. I have also shown that particles of the diseased organs or artificial cultures of this bacillus when inoculated into pigs, mice and rabbits, produce the disease with multiplication of the bacillus, while pigeons, fowls, and guinea-pigs are unaffected by cultivations of the bacilli or by the original virus.

Pasteur maintains that in an infectious disease of the pig known in France as rouget and chiefly characterised by red patches of the skin there is present in the blood a dumb-bell

micrococcus, which, when artificially cultivated yields material which, inoculated into pigs, is said to produce the disease. Mice, rabbits, and pigeons are susceptible to the disease, but in rabbits after several transferences the virus becomes attenuated, and with this a mild form of the disease can then be produced, protecting the animal thus operated upon from a subsequent severe attack.

From the recent investigations on this subject by Schütz[1] it is quite clear, that the disease that in France is called rouget and in Germany erysipelas of swine, is an altogether

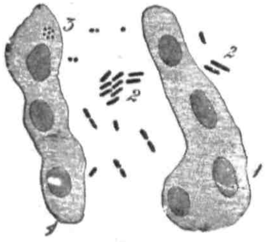

FIG. 64.—FROM A PREPARATION OF BRONCHIAL MUCUS OF A PIG DEAD OF SWINE PLAGUE.

1. Detached epithelial cells of alveoli.
2. Bacilli.
3. Micrococci.

Magnifying power 700. (Stained with Spiller's purple.)

different disease from what in this country and America is called swine-fever, or swine-plague, or pig-typhoid. The symptoms and course of the disease are totally different in the two maladies. Schütz has shown that the disease rouget or erysipelas is due to a minute bacillus which in size, mode of cultivation, and effect on inoculation, is almost identical with the bacillus of mouse-septicæmia (Koch). Schütz examined some of the attenuated lymph sent by M. Pasteur,

[1] *Mitth. aus d. k. Gesundheitsamte*, Berlin, i. 1885.

and found that it contained the dumb-bell micrococcus described by M. Pasteur; but this was accidental contamination; there was present the minute bacillus, and the action of such lymph was due not to the micrococcus but to this bacillus.

Artificial cultivations made in broth and hydrocele fluid from diseased organs of the pig, mouse, and rabbit, after an incubation of twenty-four hours at temperatures ranging between 30° and 42° C. contain the above rods, which crowd

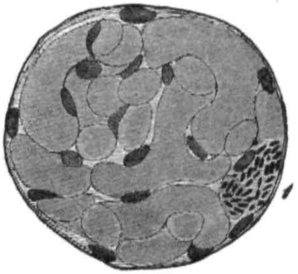

FIG. 65.—FROM A SECTION THROUGH THE KIDNEY OF RABBIT DEAD OF SWINE PLAGUE, SHOWING A MALPIGHIAN CORPUSCLE, THE CAPILLARIES OF THE GLOMERULUS BEING TRANSFORMED INTO HYALINE IMPERMEABLE CYLINDERS.

1. Bacilli.
Magnifying power 500. (Stained with Spiller's purple.)

the nourishing fluids, all being rather short, about 0·002 to 0·003 mm. long, and all possessed of the power of active locomotion, such as is known to be possessed by the septic bacterium termo and bacillus subtilis. During the following days of incubation, while the rods multiply, many of them lose their motility, grow longer, up to 0·005 mm. and more, and in some of the longer samples bright spores make their appearance, one spore at one or both ends or sometimes in the centre.

From these cultivations new cultivations may be made and carried on through successive generations, all cultures behaving in the same manner; and in all of them the rods only are present, and show exactly the same changes as in the parent culture.

The smallest droplet of any of these cultivations produces the disease in pigs, mice, and rabbits. The mice and rabbits die with exactly the same appearances and with the same

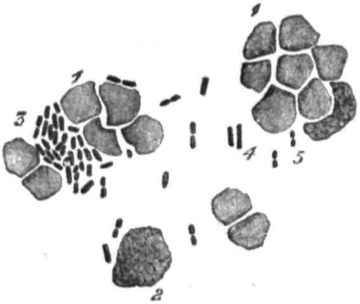

FIG. 65.—BLOOD OF FRESH SPLEEN OF A MOUSE THAT DIED OF SWINE PLAGUE.

1. Blood discs.
2. A large nucleus.
3. Groups of minute bacilli.
4. Long bacilli.
5. Dumb-bells of bacilli.

Magnifying power 700. (Stained with gentian violet.)

anatomical lesions as when they are inoculated with material directly taken from the diseased organs of a pig dead of swine plague. Those animals generally die on the fifth, sixth, or seventh day, and on post-mortem examination show a characteristic swelling of the spleen, a characteristic disease of the liver (chiefly coagulative necrosis of tracts of the liver tissue), and inflammation of the lungs.

Inoculations of suitable sterilised nourishing fluids made

from the spleen, liver, and lung of such animals always result in producing a copious crop of the characteristic bacilli, as

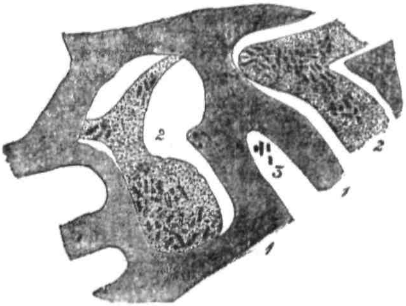

FIG. 67.—FROM A SECTION THROUGH A NECROTIC PATCH OF THE LIVER OF A MOUSE DEAD OF SWINE PLAGUE.

1. Tracts of liver cells shrunk.
2. Capillary blood-vessels filled with very small micrococci, amongst which are seen the bacilli.
3. Bacilli only.

Magnifying power 700. (Stained with Spiller's purple and magenta.)

do those made with the lung and bronchial glands of pigs dead of swine plague; but from the blood of the pig the cultivations do not as a rule succeed, nor as a rule from the

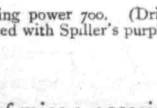

FIG. 68.—BACILLI OF SWINE PLAGUE, FROM AN ARTIFICIAL CULTURE, AFTER FORTY-EIGHT HOURS' INCUBATION.

Magnifying power 700. (Dried and stained with Spiller's purple.)

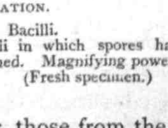

FIG. 69.—BACILLI OF SWINE PLAGUE, FROM AN ARTIFICIAL CULTURE, DURING SIXTH DAY OF INCUBATION.

1 and 2. Bacilli.
3. Bacilli in which spores have been formed. Magnifying power 700. (Fresh specimen.)

blood of mice; occasionally, however, those from the blood of rabbits dead of the disease do succeed.

Quite recently I have ascertained that pigs inoculated with artificial cultures of these rods (started from the pig, mouse, or rabbit dead of the plague) or with the diseased organs of a mouse or rabbit, suffer from a mild form of the disease, which after one or two weeks passes off completely. I have had pigs that had been twice inoculated, the first time with artificial cultures, the second time with diseased organs of mouse and rabbit, and each time the pigs suffered from a mild form of the disease. They were then inoculated a third time with the juice of the diseased (fresh) lung of a pig dead of the plague; this time also they showed distinct symptoms of the disease, but after a few days to a week they completely recovered. If normal (or not previously inoculated) pigs are inoculated with matter from the diseased fresh lung of a pig dead of the plague, they as a rule die from a virulent form of the disease. But in the above case they were protected by previous inoculations, not altogether against a new attack but against a fatal attack.

(*i*) *Bacillus Lepræ.*—Armauer Hansen[1] first ascertained the existence of large numbers of minute bacilli in the peculiar large leprosy-cells of Virchow, which occur in the nodules of leprous patients. Neisser confirmed this, and considerably extended our knowledge of the bacilli, showing that they can be readily stained pink with fuchsin or with Ehrlich's acid solution of eosin-hæmatoxylin. The bacilli are fine rods about 0·004 to 0·006 mm. long and less than 0·001 mm. thick. They are pointed at their ends, and always occur in masses within the large leprosy-cells of the leprous tubercles of the skin and internal organs. But they

[1] *Virchow's Archiv*, vol. lxxix.; and *Quart. Journ. of Micro. Sci.* 1880.

are also present in the interstitial tissue of the nervous branches in the anæsthetic variety of the disease.[1] Some bacilli are motile, others not; some possess bright oval spores, and others are more or less beaded, owing to local collections of the protoplasm within their sheath. Neisser and Armauer Hansen have cultivated them artificially in

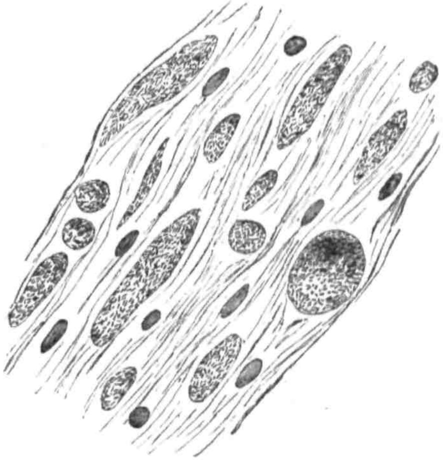

FIG. 70.—FROM A SECTION THROUGH THE LARYNX OF A PATIENT DEAD OF LEPROSY.

Huge cells in fibrous connective tissue; the cells are filled with the leprosy bacilli.
Magnifying power 600. (Stained with magenta and vesuvin.)

blood-serum and in solutions of meat-extract. Neisser has also shown that the characteristic leprosy-cells are only wandering cells modified by the growth and multiplication in them of the bacilli. In the blood the bacilli do

[1] Compare also Cornil, *Union Médicale*, 1881, Nos. 178, 179, and Babes, *Archives d. Physiologie*, July, 1883.

not occur, and they spread probably only by way of the lymphatics.

Inoculation experiments on domestic animals and monkeys have hitherto failed.[1] Damsch[2] maintains, however, that

FIG. 71.—BACILLI OF THE SAME PREPARATION AS IN PRECEDING FIGURE. More highly magnified, 1000.

he was able, by inoculation with leprous tissue into the peritoneal cavity and into the skin, to produce in cats a

FIG. 72.—CELLS OF THE LEPROSY NODULES OF MAN, FILLED WITH THE LEPROSY BACILLI (AFTER NEISSER).

distinct increase and sprouting of the bacilli. Preparations of leprous nodules of the larynx and skin made by my

FIG. 73.—FROM AN ARTIFICIAL CULTURE OF BACILLUS OF LEPROSY (AFTER NEISSER).

friend, Mr. A. Lingard, and stained with Weigert's solution of magenta and vesuvin, showed the leprosy-bacilli completely filling all the cells, small and large, spherical and

[1] Köbner, *Virchow's Archiv*, vol. lxxxviii.; Hansen, *ibidem*, vol. xc.
[2] *Virchow's Archiv*, vol. xcii.

spindle-shaped, contained between the connective-tissue bundles.

In a section through the liver of a bird (*Rhea*) that died in the Zoological Gardens in London, prepared by Dr. Gibbes after his method of staining for tubercle-bacilli, there were seen innumerable aggregations of larger and smaller pink masses (visible to the unaided eye as dots of the size of a pin's point to that of a pin's head or millet-seed, and

FIG. 74.—FROM A SECTION THROUGH A NODULE OF THE LIVER OF RHEA.
1. Cells of various sizes filled with minute bacilli; owing to the smallness of the bacilli and to their being crowded in the cells and owing to the comparatively low magnifying power (300) the bacilli appear like dots.
(Stained with fuchsin and methyl-blue.)

larger). Under the microscope these pink masses were seen to be composed of cells of various sizes, each filled with an enormous number of what appeared under a high power very short bacilli, much shorter than tubercle-bacilli. But they gave the same reaction as tubercle-bacilli. Here and there isolated cells of various sizes could be seen filled with the bacilli. In the large cells the cell-outline was becoming

indistinct, and in some the cell-substance was seen to break down, whereby the bacilli became free. In these respects, in the size, distribution, and character of the bacilli, there exists a remarkable similarity between the nodules in leprosy and the nodules just mentioned.

FIG. 75.—TWO CELLS OF THE LEPROSY (?) NODULES IN THE LIVER OF A BIRD (RHEA).

The cell-substance is crowded with minute bacilli, similar to leprosy-bacilli Magnifying power 700. (Stained with magenta.)

(*j*) *Bacillus of malignant œdema* (Koch), *vibrion septique* (Pasteur). By inoculating mice, rabbits, and especially guinea-pigs subcutaneously with a comparatively large quantity of earth, or of putrid fluid, one occasionally produces death in twenty-four to forty-eight hours. This form of septicæmia is also called "Pasteur's septicæmia," and is of course distinct and different from Davaine's septicæmia.[1] At the seat of the inoculation and spreading from it into the subcutaneous tissue of adjoining parts there is much discoloration and occasionally hæmorrhage ; a turbid offensively-smelling ichor fills the spaces of the subcutaneous tissue, and in it are found large numbers of bacilli, some motile, others not. The lungs are hyperæmic

[1] Rosenberger maintains (*Centralblatt f. d. med. Wiss.* 4, 1883) that the blood and exudation-fluids of rabbits dead of Davaine's or Pasteur's septicæmia can be effectually sterilised by heat without losing their specific action, reproducing on injection into fresh animals the disease with the recurrence of the organisms characteristic of the disease. Dowdeswell, however, states (*Proceedings of the Royal Society*, 221, 1882) that this is not the case, for on really effectual sterilisation by heat the organisms are killed, and the fluids become innocuous.

and have small hæmorrhagic spots. The spleen is invariably enlarged and hæmorrhagic spots are often noticed on the peritoneum of the abdominal organs, and there is some peritoneal exudation. The blood of the spleen, of the liver, lung, and intestine, the serous coating of the abdominal organs, and the peritoneal exudation, contain the same bacilli as the subcutaneous exudation. Many of them

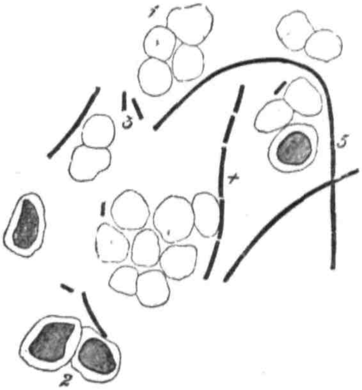

FIG. 76.—BLOOD OF A GUINEA-PIG DEAD OF KOCH'S MALIGNANT ŒDEMA.

1. Red blood discs.
2. White corpuscles.
3. Single bacilli.
4. Chain of long bacilli.
5. Leptothrix.

Magnifying power 700. (Stained with gentian violet.)

include spores. By injecting the bacillus into the peritoneal cavity of guinea-pigs death is produced rapidly, especially after passing it through two generations, so rapidly indeed that the animals often die within sixteen hours (Burdon Sanderson and Klein). In all these instances a viscid transparent slightly but spontaneously coagulable exudation, poor in white and red corpuscles, is found in the peritoneal

cavity, and the peritoneum of all parts is highly inflamed. Bacilli are present in it in enormous numbers, many of them containing spores. The blood of the heart does not contain bacilli immediately after death, but has them some hours after.

The bacilli in question are about 0·003 to 0·005 mm. long and a little over 0·001 mm. thick; they are rounded at their ends; they form chains of two and more, and these chains are straight or broken. They also form leptothrix, straight, or more commonly curved. The bacilli have been artificially cultivated by Pasteur [1] in blood-serum and in neutral solution of Liebig's meat-extract. Gaffky [2] grew them on potatoes at 38° C. The artificial culture is capable of producing the malignant œdema, but it is always necessary to inject more than minimal quantities. The bacilli grown in fluids outside and inside the body form spores without free supply of air, and are therefore anaërobic (Pasteur).

In human fæcal matter there are always present innumerable masses of bacteria—micrococci, single and dumb-bells, and in clumps of zooglœa, bacterium termo, and various species of bacilli, varying in thickness, length, and in motility, some being motile, others not. It has been shown by Bienstock (*Centralbl. f. med. Wiss.* 1883, p. 949) that a bacillus can be cultivated from normal human fæces which in many respects resembles the bacillus of malignant œdema; it produces death in mice, but without the symptoms of malignant œdema.

Professor Rossbach has maintained (*Centralblatt f. d. med. Wiss.* 5, 1882) that when a solution of papayotin (the juice of *Carica papaya*) is injected into the veins of a rabbit, the animal dies, and shortly after death—even so short a time as fifty minutes after the injection—there are found in the blood large numbers of bacteria. Dowdeswell, however, states (*Practitioner*, May, 1883) that solutions of papayotin contain as a rule the spores of a motile bacillus which in all respects resembles bacillus subtilis; in artificial cultures in 10 per cent. solutions of papayotin, in blood-serum, and in broth, these spores develop into

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[1] *Bull. de l'Acad.* 1877. [2] *Mittheil. a. d. k. Gesundh.* 1880.

bacilli which form leptothrix filaments, and in them spores soon make their appearance. Filtered papayotin solutions, when injected into the blood of rabbits, kill like unfiltered ones, but neither during life nor after the death of the animals could any organisms be detected in the blood. It appears to follow from these experiments, that papayotin solutions contain spores, and that these spores are those of a bacillus subtilis which does not possess any specific pathogenic properties.

(*k*) *Bacillus of symptomatic anthrax* (Ger. *Rauschbrand*, Fr. *charbon symptomatique*, Arloing, Cornevin, and Thomas, *Bull. de l'Acad.* 1881; Eng. *black leg, quarter-evil*). This

FIG. 77.—BLOOD OF A GUINEA-PIG DEAD OF SYMPTOMATIC ANTHRAX.

Blood-corpuscles and between them several bacilli. Magnifying power 700. (Stained with Spiller's purple.)

disease, which is not uncommon in cattle, generally ends fatally and is very infectious. It is characterised by hæmorrhagic effusion (or "tumour") in the subcutaneous and intermuscular tissues of one or other, or both, anterior or posterior extremities, in consequence of which the movements of the animal so affected become greatly impeded. The animals generally die in the course of the second or third day after infection. The subcutaneous tumour contains numerous bacilli, as do the abdominal and thoracic viscera.

The bacilli are about the size of those of malignant anthrax

0·02 mm. in length, and 0.001 to 0·0012 in thickness; they are truncated.[1] The spores produced by growing the bacilli

FIG. 79.—PART OF A BLOOD CLOT FROM THE HEART OF A MOUSE DEAD OF ANTHRAX.

Magnifying power 500. (Stained with Spiller's purple.)

with free access of air are about 0·001 mm. thick, and about 0·002 to 0.003 mm. long. They are not stained by the ordinary dyes and differ herein from the bacilli.

In the human subject malignant anthrax occurs as "woolsorter's disease"; for the ætiology and pathology of this malady see Spears (*Reports of the Medical Officer of the Local Government Board*, 1881 and 1882) and Greenfield (*ibid.* 1881). It occurs also in sorters of hides.

All rodents and herbivorous animals are susceptible to anthrax; rats are, however, infected with difficulty, pigs are very insusceptible, and so are dogs and cats. Infection of animals can be produced by inoculation into the skin and subcutaneous tissue, intravascular injections, and by inhalation of spores (Buchner, *Untersuchungen über niedere Pilze*, by Prof. v. Nägeli, 1882, p. 178). In woolsorter's disease the usual mode of infection is by inhalation of spores adhering to the wool of the fleeces of animals (sheep, goats) dead of anthrax. As in rodents infected with anthrax, so also in man, the blood-vessels of all organs contain the bacilli, and extravasations of the infected blood are frequent

[1] It is generally assumed that the bacilli are the same in all animals affected with splenic fever, but this is most undoubtedly not the case, as has been already pointed out by Huber (*Deutsche med. Woch.* 1881); the bacilli of the guinea-pig are thicker than those of the mouse or sheep, and these again are thicker than those of the rabbit.

in many parts of the body. The presence of bacilli in the extravasations into the mucous membrane of the trachea and bronchi does not necessarily mean that these parts represent the points of entrance of the bacilli into the system, as Greenfield seems to regard as self-evident (*Reports of the Medical Officer of the Local Government Board*, 1881). As a matter of fact I find in every lung of mouse, rabbit, and guinea-pig, dead after subcutaneous inoculation with anthrax, bacilli anthracis in the alveolar cavities and in the smaller and larger bronchi. Ingestion of bacillar material is sometimes followed by anthrax, but in these cases abrasions in the mucous membrane of the mouth, pharynx, or gut, may have been the real place of entrance. Mice fed with anthrax material do not become infected (Klein, *ibid.* 1881). But the reported cases of intestinal mycosis (see for the literature of this subject, Koch, "Ætiologie d. Milzbrandes," *Mittheil. a. d. k. Gesundheitsamte*, 1881,) seem nevertheless to indicate that such a mode of infection, namely, by the alimentary canal, is not excluded. Compare also Falk, *Virchow's Archiv*, vol. xciii. From recent observations by Koch and others, it has become clear, that infection by the alimentary canal can be readily produced with spores.

Rodents inoculated with the bacillus of the blood or spleen of an animal dead of anthrax, or with the bacillus or spores of an artificial culture, die generally within forty-eight hours; in some instances in twenty-four to thirty hours, in other exceptional instances after forty-eight to sixty hours. The blood in all instances contains the bacilli, the spleen is large and full of bacilli, and so are the blood-vessels of most other organs, the exudations, and the urine. In the placenta of a pregnant guinea-pig dead in consequence of inoculated anthrax, I have seen that the bacilli kept strictly as a rule within the maternal blood-vessels, and are wholly absent in the blood of the vessels of the fœtus. Subcutaneous inoculation or injection into the cutis of the minutest quantity of bacillus-containing material (blood or artificial culture) invariably produces death. Subcutaneous injection of bacillus-containing material in the guinea-pig almost always produces a characteristic œdema, spreading sometimes over

a large area. The œdematous fluid is clear and contains only a few bacilli.

Archangelski (*Centralblatt f. d. med. Wiss.* 1883, p. 257) claims to have ascertained that if an animal be inoculated with anthrax, many hours before the bacilli appear in the blood, there are present numbers of spores. Just before death they all become changed into the bacilli. He further maintains that those spores taken from the blood can be shown to multiply by division, and without changing into bacilli, by cultivating them artificially with exclusion of oxygen. I have shown, however (*Reports of the Medical Officer of the Local Government Board for* 1883), that none of these assertions are borne out by actual observation, and that they are erroneous.

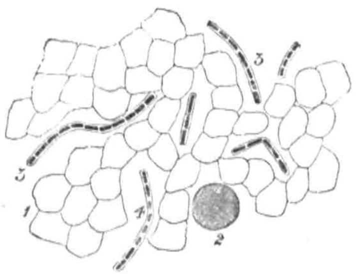


FIG. 80.—FROM A PREPARATION OF HEART'S BLOOD OF A GUINEA-PIG DEAD OF ANTHRAX.

1. Red blood-discs.
2. White corpuscle.
3. Bacilli anthracis.

Magnifying power 700. (Stained with Spiller's purple.)

Any fluid containing proteid material is a suitable nutrient medium for the bacilli; they grow abundantly at all temperatures between 15° and 43° C., best between 25° and 40° C. They elongate and divide rapidly, and the bacilli grow out into long curved and peculiarly twisted filaments which often form bundles, the individual filaments being twisted round one another like the strands of a cable.

The bacillus anthracis grows best in neutral fluids, and to

a lesser extent also in alkaline fluids which contain proteid material. When growing in neutral nourishing fluids, it forms on the bottom of the fluid characteristic fluffy whitish masses, which are convolutions of the characteristic filaments. These appear homogeneous in the fresh state, their ends being slightly thicker and rounded. Examined in preparations made after the Weigert-Koch method (*i.e.* drying of a thin layer and staining it with aniline dyes, washing in

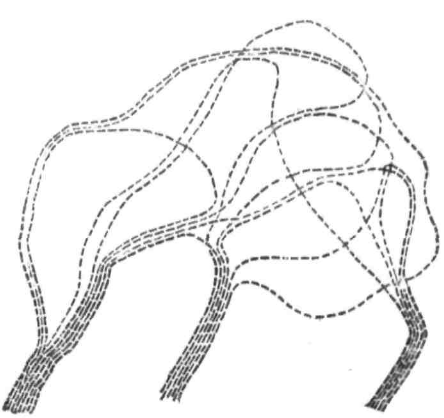


FIG. 81.—FROM AN ARTIFICIAL CULTURE OF BACILLUS ANTHRACIS.

Convolutions of threads, each composed of bacilli.
Magnifying power 300. (Stained with Spiller's purple.)

water, then in spirit, then again in distilled water, and then drying and mounting in Canada-balsam solution), all the bacilli and their filaments are seen to be composed of a thin hyaline sheath, and in this is a row of cubical or rod-shaped masses of protoplasm taking the dye very readily. According to the length of the bacilli the number of these *elementary masses of protoplasm* varies. Some of the rod-shaped

elements appear constricted in the middle, preparatory to division. Between each two elements is a fine septum.

Bacilli anthracis when growing at ordinary temperatures on a solid medium (*e.g.* a mixture of gelatine and broth, or Agar-Agar and peptone) show a very peculiar modification, inasmuch as some of the elements assume a spherical or oval

FIG. 82.—FROM AN ARTIFICIAL CULTURE OF BACILLUS ANTHRACIS, CARRIED ON AT ORDINARY TEMPERATURE AND ON SOLID (GELATINE) MATERIAL. TORULA FORM.

Magnifying power 450. (Stained with Spiller's purple.)

shape, a torula-form, and as such they multiply by gemmation and division, and form clusters or arrange themselves in chains. By and by each of these spherical elements elongates into a rod, and when all elements have undergone this change we have the typical smooth filament of the

leptothrix form. Some of the elements in such a filament remain for a long time of a spherical shape, and are much larger, looking like the sporangium of a nostoc-alga. The most interesting forms are those where an ordinary smooth filament of anthrax-bacillus at its growing ends shows itself to be composed of a chain of torula-elements. Such torula forms occur also in ordinary cultivations in fluid media at temperatures of 20° to 30° C., but not by any means so often as at ordinary temperatures and in a solid medium. These torula-cells are about 0·0013 to 0·0026 mm. in diameter. The torula-forms are very virulent, but in an animal always assume the ordinary shape of the typical bacillus.[1]

As has been mentioned in treating of pigment bacilli, such a torula-form has been also observed by Neelsen in the bacillus that causes the colour of "blue milk;" and Zopf[2] has observed it also in cladothrix dichotoma.

I have also observed this torula-modification in the filaments of septic bacilli, in a bacillus that I found growing accidentally in pork-broth. The bacillus had the same morphological characters as the bacillus subtilis of hay-infusion, and also formed a pellicle composed of filaments. In some of the filaments the large torula-like cells could be seen here and there interposed between cubical and cylindrical cells.

On inoculating fluid media (*e.g.* broth of any kind or peptone fluid) with the bacilli anthracis, either those of the blood or of the spleen of an animal dead of anthrax, and shaking the fluid so as to distribute the bacilli uniformly through the fluid and exposing this to a temperature of from 25° to 40° C., it will be noticed that after twenty-four to forty-

[1] Klein, *Quart. Journ. of Microsc. Science*, April, 1883.
[2] *Zur Morphologie d. Spaltpflanzen*, ii. and *Die Spaltpilze*, Breslau, 1883.

eight hours incubation, the fluid is uniformly turbid, owing to the rapid multiplication of the bacilli. These are shorter or longer typical anthrax-bacilli. But as incubation proceeds all the bacilli grow into filaments, and these being heavier sink to the bottom of the fluid and form the characteristic whitish fluffy convolutions. But on inoculating dilute broth, care being taken that the inoculating material, whether

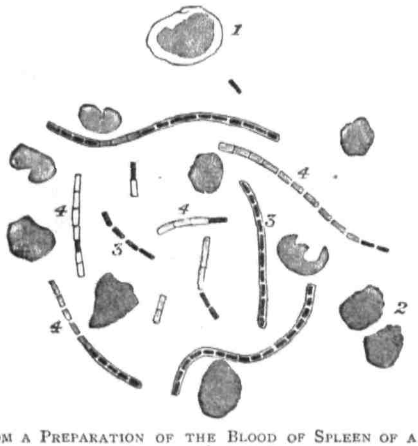


FIG. 83.—FROM A PREPARATION OF THE BLOOD OF SPLEEN OF A GUINEA-PIG DEAD OF ANTHRAX.

1. White blood-corpuscle.
2. Red blood-discs, shrunken.
3. Chains of bacillus anthracis.
4. Degenerating bacilli, the sheath only being preserved.

Magnifying power 700. (The preparation has been stained with gentian-violet.)

consisting of blood-bacilli, bacilli of a culture, or spores of culture-bacilli, is deposited at once on the bottom of the fluid, and this is not shaken up, it will be noticed on incubation that the fluid remains limpid. All the growth, in the shape of the fluffy whitish masses, takes place at the bottom.

After a few days' incubation, no matter what the temperature is, many of the bacilli and their leptothrix-filaments show signs of degeneration, consisting in the granular disintegration and absorption of the protoplasmic contents of the bacilli and their filaments, at first only here and there, but by and by over longer pieces. Such bacilli and leptothrix-filaments appear in such places as if empty. This is also noticed in the bacilli of the blood and spleen of an animal inoculated with anthrax even at the point of death or soon after death, if the number of bacilli is great.

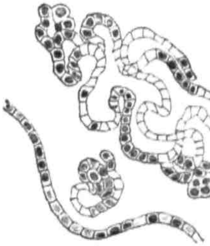


FIG. 84.—FROM AN ARTIFICIAL CULTURE OF BACILLUS ANTHRACIS IN BROTH AFTER MANY DAYS INCUBATION.

The threads are swollen and curled up, and in many places the protoplasm has disappeared, leaving the sheath and septa distinct. Magnifying power 700. (Stained with Spiller's purple.)

Another form of degeneration consists in the filaments of bacilli becoming much curled and swollen, and finally disintegrated into an amorphous débris.

As long as the bacilli grow in the depth of a fluid they never form spores, but when grown on the surface with free access of air, or on solid media (*e.g.* serum gelatine, gelatine broth, Agar-Agar, potato, &c.), the bacilli, having developed into filaments, proceed to form spores. But they may form spores even in fluid media if by some accident, either by

sticking to the glass vessel containing the fluid or by means of a cotton-wool fibre, some of the bacilli remain on the surface of the fluid. This formation of spores is not due to exhaustion of the nourishing medium, as is maintained by Buchner—it has, in fact, nothing to do with it—but represents the last stage in the life-history of the bacilli, provided they have an ample supply of oxygen. If this latter condition is not fulfilled, as when they are grown at the bottom of a fluid, the bacilli gradually degenerate as mentioned above.

Spore-formation occurs, *cæteris paribus*, at all temperatures between 18° and 45° C. Koch found 15° C. the lower limit. Pasteur states that in a nutrient medium exposed to a temperature of 42° to 43° C. the bacilli are not capable of forming spores; but this is not correct, for when the bacilli are growing on the surface of the nutrient medium, they form spores even at a temperature of 44° to 44°·5 C., as I have conclusively shown by growing them on Agar-Agar and peptone mixture. The spore-formation consists in the appearance of a bright glistening spherical body in the protoplasm of an elementary mass or cell; this body gradually enlarges till it reaches its full size, becoming at the same time oval. The bacilli at these points are thicker than where no spore-formation has set in. Under the most favourable conditions, each cubical or rod-shaped mass of protoplasm includes one spore, in which case the bacillar filament contains an almost unbroken row of spores; but in other cases only an elementary mass here and there contains a spore, the rest breaking down and becoming absorbed. In the first case also, the protoplasm of the elements almost entirely disappears, the sheath swelling up and becoming hyaline, and only the bright spores remaining. Their linear arrangement, however, still indicates that they were formerly contained in one filament.

If bacilli grow in the depth of a fluid medium, they do not form spores, as has been stated above; and as we have also seen, as new bacilli appear, or the old filaments increase in length, degeneration sets in. This degeneration gradually affects greater and greater numbers, and when the fluid is

FIG. 85.—FROM AN ARTIFICIAL CULTURE IN NEUTRAL PORK-BROTH OF BACILLUS ANTHRACIS, WITH COPIOUS FORMATION OF SPORES.
Magnifying power 700. (Stained with Spiller's purple.)

exhausted for the formation of new bacilli, it necessarily follows that the whole growth gradually becomes involved in the process of degeneration, the whole mass becoming smaller, and finally only débris is left. Such cultures, namely, those in which the degeneration involves the whole

mass of the bacilli, are quite innocuous when inoculated into animals, or into fresh nourishing media. But as long as there are any good protoplasmic elements of the bacilli left, the culture is virulent to rodents, with the exception of mice, as will be stated presently; and it is capable, when transferred to new suitable nourishing media, of starting new cultures that prove virulent to all rodents and sheep.

The same holds good of the bacilli in the blood and organs of an animal dead of anthrax, provided the animal be not opened, and its organs, exudations, or urine be not exposed to the free air; for the bacilli not exposed to the air gradually degenerate, and the blood and organs of such an animal although at first deadly poison to other susceptible animals, become at length quite innocuous. Systematic observation has shown me that small animals, such as mice and guinea-pigs, when kept unopened or buried in earth, become quite innocuous after five to eight days, the anthrax-bacilli having by this time, by degeneration, altogether disappeared from the blood, spleen, and other organs. Pasteur's statement that in animals dead of anthrax and buried, the bacilli form spores, that these spores are taken up by earthworms and carried to the surface of the soil, where they are deposited with their castings and thus are capable of infecting animals grazing or sojourning on this soil, is not borne out by the above observations. And further, Koch has proved[1] by direct experiment that spores of anthrax-bacilli when mixed with earth in which worms are present, are not taken up by these creatures.

Drying bacilli of the blood or of a culture in a thin layer invariably kills them, but the spores remain unaffected.

The bacilli of the blood of a rodent dead of anthrax are

[1] *Mittheil. a. d. k. Gesundheitsamte*, 1881.

always thinner than the bacilli cultivated in a neutral fluid medium like pork-broth.

Cultivation of the blood-bacilli at temperatures varying between 20° and 40° C. in any suitable nourishing material, solid or fluid, however many transferences (new cultivations or so-called new generations) be made, always yields a crop of virulent bacilli. It is absolutely incorrect to say, as Buchner[1] and Greenfield[2] maintain, that continued transference weakens the action of the bacilli; as long as the cultures remain pure, not contaminated and finally suppressed by accidental innocuous bacilli, the anthrax-bacilli retain their full virulence.

Cultures of the blood-bacilli at 20° to 38° C. in fluid media, *e.g.* neutral pork-broth, during the first or second week, are virulent to mice, guinea-pigs, and rabbits; but after that they lose their power on mice, provided the growth takes place only in the depth and no spores are formed; but they retain it, as regards guinea-pigs and rabbits, as long as they contain good bacilli at all.[3] But fresh cultures made of such bacilli invariably produce a growth which is fatal to all rodents during the first or second week.

Pasteur has stated that blood-bacilli which have become attenuated in virulence by exposure to 42° or 43° C. for twenty days are capable of starting new cultures of attenuated virus. This I question, for I find that such a culture starts new cultures of virulent bacilli; in the same way the bacilli of a culture that is only "vaccine" for sheep, when it is inoculated into a guinea-pig kills it with anthrax, and then yields bacilli that are fatal to sheep.

[1] *Ueber d. Erzeug. des Milzbrandes*, Munich, 1880.
[2] *Proceedings of the Royal Society*, June 17, 1880.
[3] Klein, *Reports of the Medical Officer of the Local Government Board*, 1881.

Blood-bacilli exposed to a temperature of 55° C. or to a solution of ½ to 1 per cent. of carbolic acid, lose their virulence (Toussaint). Chauveau found that exposure to a temperature of 52° C. for fifteen minutes, or of 50° C. for twenty

FIG. 86.—NETWORK OF CAPILLARIES FILLED WITH BACILLUS ANTHRACIS; FROM THE OMENTUM OF A RABBIT DEAD OF ANTHRAX.

1. Extravasation of the bacilli.
2. Capillaries filled with the bacilli.
Magnifying power 350.

minutes, destroys the virulence of the blood-bacilli. Pasteur[1] ascertained that by cultivating blood-bacilli in chicken-broth at 42° to 43° C. they lose their virulence after twenty days'

[1] *Comptes Rendus*, 1881; *Transactions of the International Medical Congress in London*, 1881, vol. i.

cultivation, not as Pasteur thinks owing to the action of oxygen, but owing to the high temperature; and when such bacilli are injected into sheep and cattle they do not kill though they induce sometimes a slight illness. After this illness has passed off, the animals are protected against virulent anthrax. But with reference to this "vaccination," it must be borne in mind that twenty days' cultivation of blood-bacilli at 42° to 43° C. does not always yield attenuated virus, [1] and also that sheep and cattle not killed by inoculation of attenuated virus produced by Pasteur's method [2] or by other means (see below), although they are protected against virulent anthrax, remain so only for a limited time, probably about nine months.

In all these experiments with the anthrax-bacilli it is necessary to bear in mind that by passing the bacilli through different species of animals they become endowed with different qualities, and that bacilli which are fatal to some are not fatal to all animals. While, for instance, the blood-bacillus of sheep or cattle dead of anthrax invariably produces death when inoculated into sheep or cattle, after passing through white mice [3] it loses this virulence for sheep and cattle. The blood of white mice dead of anthrax does not kill sheep; it produces only a transitory illness and the animals are, for a time at least, protected against virulent anthrax. The blood of guinea-pigs dead of anthrax produces illness, sometimes death, in cattle, but as a rule does not kill (Sanderson and Duguid), and the blood of the biscachia of

[1] Klein, *Reports of the Medical Officer of the Local Government Board*, 1882.

[2] Pasteur thinks that such cultures remain free of spores because of the temperature of 42°—43° C.; but this is not so, as has been pointed out above; the statement only holds good so long as the bacilli are prevented from growing on the surface.

[3] Klein, *Reports of the Medical Officer of the Local Government Board*, 1882.

South America does not kill cattle, while it gives them a transitory illness, and after this immunity for a time.[1] Again,

FIG. 87.—FROM A SECTION THROUGH THE KIDNEY OF A RABBIT DEAD OF ANTHRAX.

The capillaries of the cortex are naturally injected with the Bacillus anthracis.
1. A glomerulus.
2. Capillaries surrounding the convoluted uriniferous tubules not shown here.
Magnifying power 450. (Spiller's purple.)

Pasteur's "vaccine," which as a rule (but not without exception) does not kill sheep or cattle, is fatal to ro-

[1] Roy, *Nature*, December, 1883.

dents.[1] From all this it follows that as regards virulence the bacilli anthracis differ in the different species of animals, and in them acquire different qualities. A culture that does not kill mice, such as an artificial culture of blood-bacillus after one or two weeks' incubation at 20° to 35° C., or a culture that for other reasons, as when attenuated by heat or antiseptics, does not produce fatal anthrax in guinea-pigs, fails to give to these animals any immunity whatever. Rodents, so far as my experience goes, either die of inoculation with anthrax-bacilli or they do not die; but they are not provided with immunity by the attenuated virus.

Koch[2] maintains that in neutral chicken-broth the bacilli growing at 42° C. lose their virulence in thirty days, and at 43° C. in six days, first for rabbits, then for guinea-pigs, and lastly for mice. I am quite sure from my own observations, that these results are not uniformly obtained, since I have seen anthrax-bacilli very virulent both for rabbits and guinea-pigs even after growing for thirty-six days at 42°·5 C.

Bacillus anthracis is capable, as we have seen, of growing well outside the body, and, when well supplied with oxygen from the air, of forming spores which represent the permanent seeds. Thus if animals, such as sheep and cattle, die of anthrax in a field, the bacilli in the effusions of such animals (*e.g.* urine, blood, effluvia from the mouth and nostrils) always contain numbers of the bacilli, and these will be able to grow indefinitely on the surface of the soil, there being always present a large amount of suitable nourishing

[1] Klein, *Reports of the Medical Officer of the Local Government Board*, 1882. Similar results have been obtained by Gaffky (*Mittheil. a. d. k. Gesundheitsamte*, 1882).

[2] *Ueber d. Milzbrandimpfung*, 1882.

Spores of bacillus anthracis stand heating to 100° C. in the dry state for over an hour without being killed; in the moist state, *e.g.* exposed to steam at 100° C., they are killed after fifteen minutes' exposure (Koch).

material, as vegetable and animal decaying matter, and since free access of air is always insured they will eventually form spores. Such soils, owing to the presence of these spores, will remain a permanent source of infection to sheep and cattle sojourning on them (Koch).

(*m*) *Bacillus tuberculosis* (Koch).—In all cases of tuberculosis in man, cattle (Perlsucht) and monkeys, of tuberculosis

FIG. 88.—FROM A PREPARATION OF HUMAN TUBERCULOUS SPUTUM, STAINED AFTER THE EHRLICH-WEIGERT METHOD.

The nuclei are stained blue, the tubercle-bacilli pink. Magnifying power 700.

artificially produced (by inoculation with human or bovine tuberculous matter) in cats, guinea-pigs, rabbits, and rats, and in spontaneous tuberculosis in birds (hens), Koch [1] found in the fresh state, and particularly after staining with methylene-blue and vesuvin, peculiar fine bacilli, some with bright oval spores, some without, some smooth and homogeneous-looking, others more of a beaded appearance. One cubic

[1] *Berliner klin. Wochenschrift*, xv. 1882.

centimetre of a concentrated alcoholic solution of methylene-blue is mixed with 200 ccm. of distilled water; to this are added two ccm. of a 10 per cent. solution of caustic potash. In this solution the fresh or hardened sections or particles of tubercles are kept for half an hour if heated up to 40° C., or for twenty-four hours if not heated. After this the preparation is stained for two minutes in a filtered concentrated watery solution of vesuvin, then washed in distilled water. On examination with a $\frac{1}{12}$ oil immersion lens and Abbé's condenser it will be found that all the elements are stained brown with vesuvin except the bacilli, which are blue. A still more successful and more delicate reaction is shown by the bacilli if the preparation is stained after Ehrlich's method. About 5 ccm. of pure anilin (anilin oil) are well mixed with 100 ccm. of distilled water and filtered; to this is added a saturated alcoholic solution of fuchsin, and with this the preparation is stained for a quarter to half an hour. It is then washed for a few seconds in a mixture of one part of nitric acid and two parts of water, and then is well washed in distilled water. The preparation when now examined shows no trace of colour except in the tubercle-bacilli, which retain the red colour of the fuchsin. The tissue may now be stained either with vesuvin or methylene-blue, which makes the ground-work brown or blue, but the bacilli remain red. This reaction after washing with nitric acid is exceedingly delicate, and is perfectly characteristic and trustworthy, as all putrefactive organisms become discoloured by the washing with nitric acid, the tubercle-bacilli only retaining the colour. There are other methods which are very good; those of Weigert and of Gibbes [1] are very quick and trustworthy in their action.

Weigert has devised a staining fluid which gives very beautiful results and is very useful for staining sections, fresh or

[1] *Lancet*, August 5, 1883.

hardened; it is as follows:—Take of a 2 per cent. watery solution of gentian-violet 12 ccm., and of a saturated watery solution of anilin oil 100 ccm. Mix. This is used like an ordinary staining-fluid for the first stain. For the second or contrast stain the following solution is used:—

Bismarck brown	1 gramme.
Spiritus vini rectificati (sp. gr. ·830) .	10 ccm.
Distilled water	100 ccm.

The sections remain in a few drops of this solution for fifteen minutes. This method yields the finest specimens of

FIG. 89.—FROM A PREPARATION OF CASEOUS MATTER FROM PULMONARY DEPOSITS IN BOVINE TUBERCULOSIS, STAINED AS IN PRECEDING FIGURE. Magnifying power 700.

tubercle-bacilli in sections through tuberculous growths that I have seen; unfortunately the colour of the bacilli is very liable to fade.

In the case of tuberculous sputum, or similar matter, a small droplet or particle is spread out in a thin layer on the cover-glass, well dried by passing it over the gas-flame of a Bunsen burner, and then stained in the way described in Chapter I. Sections of tubercles, fresh or hardened, are stained without first drying.

In all cases of human tuberculosis, particularly in the sputum, in caseating scrofulous glands, in bovine tubercles, in artificially-induced tubercles and caseating glands of rodents, the tubercle-bacilli have been shown to exist. They are most numerously found in the caseous masses in the lung found in bovine tuberculosis. Here Koch found them not only scattered through the caseous masses, but also in the well-known giant-cells; in some cases they form a more or less regular zone in the peripheral portion of the cell. But

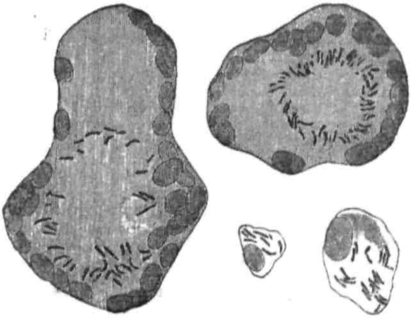


FIG. 90.—FROM A SECTION THROUGH TUBERCULOUS DEPOSITS IN THE LUNG OF A COW.
Two giant-cells and two small cells containing tubercle-bacilli. Magnifying power 700.

according to Koch the bacilli by and by disappear again from the giant-cells.

The bacilli do not show any motility and often include granules considered to be spores; they thus would be capable of forming spores within the body. Owing to these, human phthisical sputum retains its virulence even after drying. Koch cultivated the bacilli artificially, *i.e.* outside the body, and by carrying on the cultivation for several successive transmissions succeeded in isolating and clearing them from the

tuberculous tissue. These pure bacilli, no matter how many times they have been transferred, no matter how far removed from their original breeding-ground, always produced the characteristic disease when inoculated into suitable animals. The cultivation succeeded equally with material derived from human tubercles, from bovine tubercles, and from the artificially-induced tuberculosis of guinea-pigs. The bacilli grow well at a temperature varying between 37° and 39° C. in solid serum, Agar-Agar peptone mixture, and solidified hydrocele fluid.[1] (See Chapter II.) An incision is made into a tubercle with clean (overheated) scissors and a particle of a tubercle is taken up with the point of a clean (overheated) needle and deposited on the top of one of these sterile solid media kept in a test-tube plugged with sterile cotton-wool. After keeping it for ten days to a fortnight in the incubator at 37° to 39° C. the first traces of growth make their appearance in the shape of small dry whitish scales, which gradually increase in size until they coalesce. These scales are made up of the typical tubercle-bacilli lying closely side by side; some of the bacilli are longer, others shorter, and many of them have spores. New cultures may be established from these bacilli. Inoculation with them or with further cultivations into the subcutaneous tissue, peritoneal or pleural cavity of guinea-pigs and rabbits, produces after three, four, or more weeks, the typical lesions characteristic of artificial tuberculosis; namely, swollen lymphatic glands near the seat of inoculation, with subsequent caseation and ulceration; enlargement of the spleen due to numerous whitish tubercles, the larger ones caseous; enlargement of the liver, which is mottled by the presence of uniformly distributed whitish

[1] Solidified hydrocele fluid has been successfully used for the cultivation of the tubercle-bacilli, not by Koch, but by my friend Mr. Makins of St. Thomas's Hospital.

points and streaks, which by and by become confluent and caseous; tuberculosis of the peritoneum; isolated tubercles in the lungs, at first grey and transparent, then caseating in the centre; enlargement and subsequent caseation of the bronchial glands.

Owing to the fact that the tubercle-bacilli require for their growth high temperatures (37° to 39° C.), it is evident that, unlike some other pathogenic organisms, they do not thrive in the outside world in temperate climates.

Inoculation with the pure bacilli into the anterior chamber of the eye of rabbits and guinea-pigs produces the characteristic tuberculosis described by Cohnheim and Salomonsen. After an incubation of from two to three weeks there appears on the iris a crop of minute grey tubercles enlarging and undergoing caseous degeneration. Later on general tuberculosis of the eyeball and other organs follows. So that Cohnheim's assertion, that only tuberculous matter implanted into the anterior chamber of the eye can produce this outbreak of a crop of tubercles on the iris, is, by Koch's observations, strengthened in the highest degree; the tubercle-bacilli present in, and characteristic of, true tubercles are thus manifestly connected with the real cause of the morbid growth. A large number of pathologists have, since the publication of Koch's paper, devoted themselves to various parts of this question of the relationship of the tubercle-bacilli to the tuberculous process, and have, with few exceptions, verified Koch's observations. The chief opposition, leaving out of account those who, either from imperfect technical skill in the manipulation and staining of the bacilli, or by reason of the inadequate number of their observations, have denied Koch's statements, comes mainly from observers who, like Toussaint, Klebs, and Schüller, maintain that tuberculosis is due to a micro-organism which

is a micrococcus and not a bacillus, or who, like Schottelius and others, do not admit that human and bovine tuberculosis are the same, and are, therefore, not interchangeable, which they ought to be if in both the same bacillus occurs, and if this bacillus is the *vera causa morbi*. But there can be no doubt that a vast number of competent observers have fully verified Koch's dictum, that the tubercle-bacilli are specific and different from other bacilli, except those of leprosy, as regards their chemical nature (compare their behaviour to nitric acid); and that wherever they are present in the sputum we have to deal with real tuberculosis, wherever after repeated examinations they are found to be absent there is no tuberculosis. This has by this time, although not much more than a couple of years has elapsed since Koch's first publication, become in the hands of all competent workers a matter of daily practical application, especially as regards the examination for bacilli of the sputum of patients suspected of tuberculosis.

The other equally important part of Koch's discovery, namely, the artificial cultivation of the tubercle-bacilli and the production with them of tuberculosis, has also been verified by Weichselbaum.[1] Weichselbaum also ascertained [2] that in acute miliary tuberculosis of man the blood contains the bacilli.

An important series of observations was published by Mr. Watson Cheyne in the *Practitioner* for April 1883, in which he proved, (1) That the organs of rabbits and guinea-pigs suffering from the tuberculosis induced by Toussaint's cultivations from the blood of tuberculous animals, which cultivations Toussaint considered to be those of micrococci, turned out, on careful microscopic

[1] *Wiener med. Blätter*, 1883. [2] *Ibid.* 10, 1884.

examination and suitable staining, to contain the typical tubercle-bacilli; (2) That inoculations with cultures of Toussaint's pure micrococci not containing any tubercle-bacilli did not produce tuberculosis in animals; (3) That Koch's assertions as regards the constant occurrence of the tubercle-bacilli in the tubercles of animals artificially tuberculised are quite correct; (4) That material other than tuberculous does not produce tuberculosis, that is

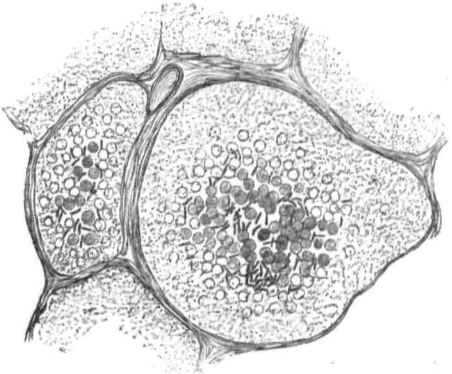


FIG. 91.—FROM A SECTION THROUGH A TUBERCLE OF THE LUNG FROM A CASE OF ACUTE MILIARY TUBERCULOSIS IN A CHILD.
Several alveoli are seen filled with debris; in the centre of this are numerous nuclei, and amongst them the tubercle-bacilli. Magnifying power about 350.

to say, that the cases of artificial tuberculosis in guinea-pigs observed by Wilson Fox and Burdon Sanderson, and in the older experiments of Cohnheim and Fraenkel, viz. those in which *chronic inflammation and caseation* (*i.e.* artificial tuberculosis) was thought to have been induced by other than tuberculous matter (*e.g.* by non-tuberculous caseous matter, setons, indifferent substances

like bits of gutta-percha inserted into the peritoneal cavity, &c.), were really due to accidental contamination with tuberculous material.

According to my own experience extending over a very large number of cases of human miliary tuberculosis and tuberculosis of cattle, I cannot for a moment accept the statement that the bacilli found in the two affections are identical; for I find that in the two diseases their morphological characters and distribution are very different. The bacilli of human tuberculosis are conspicuously larger than those of the tuberculosis of cattle, and in many instances more regularly granular. As is seen in Figs. 88-90, those of human sputum are nearly half, or at least one-third, as large again as those of the caseous masses from the lungs of cattle.

The bacilli in the tuberculous deposits of cattle are always contained in the cells; the larger the cell the more numerous the bacilli. This fact comes out very strikingly in thin and well-stained sections. Around many of the smaller and larger clumps of bacilli the cell-outline is still recognisable, and when the cell disintegrates, as it does sooner or later, the bacilli become free in groups; in this respect there exists a remarkable similarity between leprosy and bovine tuberculosis. But in the human tubercles the bacilli are always scattered between the cells.

I cannot agree with Koch, Watson Cheyne, and others, who maintain that each tubercle owes its origin to the immigration of the bacilli, for there is no difficulty in ascertaining that in human tuberculosis, in tuberculosis of cattle, and in artificially-induced tuberculosis of guinea-pigs and rabbits, there are met with tubercles in various stages—young and adult—in which no trace of a bacillus is to be found; whereas in the same section caseous

tubercles may be present containing numbers of tubercle-bacilli.

Schuchardt and Krause[1] have found tubercle-bacilli, though sparingly, in fungoid and scrofulous inflammations; Demme[2] and Doutrelepont[3] found the tubercle-bacilli also in the tissue of lupus. But the bacilli occurring in the lupus tissue, as far as I am able to see, are morphologically different

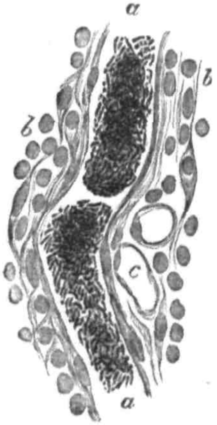


FIG. 92.—FROM A SECTION THROUGH THE KIDNEY OF RABBIT DEAD OF ARTIFICIAL TUBERCULOSIS.

a. Blood-vessel filled with caseous matter, and in it numerous tubercle-bacilli.
b. Nuclei of cells of the tuberculous new growth.
c. Capillary vessel in cross section.
Magnifying power 700.

from the tubercle-bacilli. In a preparation made of the juice of lupus-tissue, large transparent cells with several nuclei are found, in the cell-substance of which are noticed

[1] *Fortschritte d. Med.* 9, 1883.
[2] *Berliner klin. Woch.* 15, 1883.
[3] *Monatshefte f. practische Dermatologie*, 6, 1883.

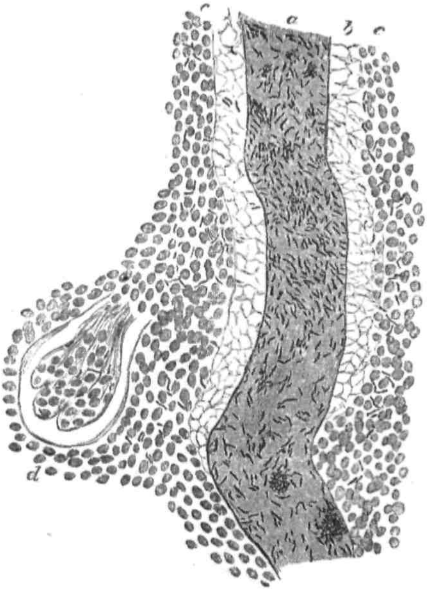


FIG. 93.—FROM THE SAME KIDNEY AS IN PRECEDING FIGURE.

a. Large artery filled with caseous matter, and in it numerous tubercle-bacilli.
b. Coat of artery.
c. Nuclei of the tuberculous new growth. *d*. A Malpighian corpuscle.

Magnifying power about 500.

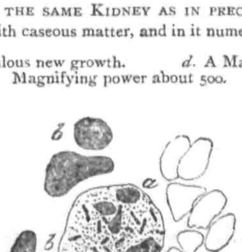


FIG. 94.—FROM THE JUICE OF LUPUS-TISSUE PREPARED AFTER THE KOCH-WEIGERT METHOD OF DRYING A THIN LAYER ON A COVER-GLASS.

Magnifying power about 700.

groups of thickish, short bacilli, thicker and shorter than tubercle-bacilli. These bacilli are either placed singly or in chains of two.

Subcutaneous inoculation of or feeding with human and bovine tubercular (caseous) matter of guinea-pigs and rabbits produces general tuberculosis, but there are certain differences both as regards the anatomical lesions as well as duration which show that the two diseases

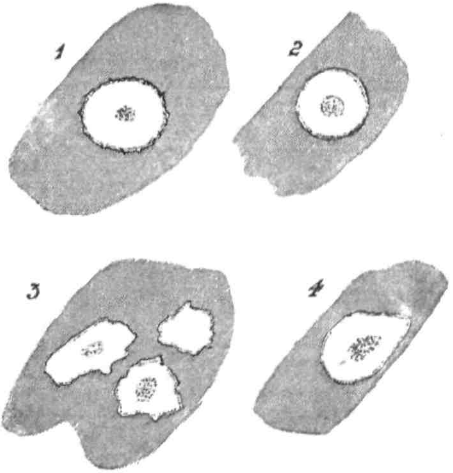


FIG. 95.—PLATE CULTIVATIONS IN NUTRITIVE GELATINE, AFTER THREE DAYS' GROWTH AT 20° C, SEEN WITH THE UNAIDED EYE.

1. A colony of micrococcus.
2, 3, 4. Colonies of cholera comma-bacilli.
The clear part is due to liquefaction of the gelatine.

are not quite identical. While rabbits are extremely susceptible to bovine tuberculosis, in consequence of inoculation or feeding, and while they develop rapidly a general tuberculosis, they are less susceptible to human tuberculosis, the disease taking a slower course and involving fewer organs, and these to a lesser degree.

It is well established that bovine tuberculosis is transmissible to pigs, cattle, sheep, monkeys, rodents, &c., by ingestion (Johne, *Deutsche*

Zeitschrift f. Thierheil, &c., 1883), but it is not experimentally proved that human beings can contract human tuberculosis by feeding on milk and flesh derived from tuberculous animals.

(*n*) *Bacillus of Syphilis.*—Lustgarten described (*Med. Jahrb. der k. k. Gesellsch. d. Aerzte.* Vienna, 1885) peculiar bacilli as occurring in syphilitic products. They resemble in size and aspect very much the tubercle-bacilli; their ends are slightly thickened, and they often show nodosities; these bacilli are never found free between the tissue elements, but always inclosed in cells, generally singly or in couples, or rarely in groups, but their total number in a given section is always small. The peculiarities they show in their mode of staining, and which they share with tubercle-bacilli and leprosy bacilli, have been mentioned in Chapter I. De Giacomi (*Schweizer Correspondenzblatt*, xv. 12) has fully confirmed the statements of Lustgarten. His method of staining the syphilis-bacilli has been mentioned in Chapter I.

Doutrelepont and Schütz (*Deutsche Med. Woch.* 1885, No. 19) have also demonstrated the occurrence of these same bacilli by simply staining sections made of syphilitic tissues in a watery 1 per cent. solution of gentian-violet with subsequent contrast staining by safranin.

On the other hand Cornil, and particularly MM. Alvarez and Tavel, state that a bacillus identical in mode of staining, size, and aspect with the one described by Lustgarten as the specific syphilis-bacillus, has been found by them in some normal secretions (*Brit. Med. Journ.* Oct. 17, 1885).

(*o*) *Bacillus of Foulbrood.*—Messrs. F. Cheshire and W. Cheyne described (*Microsc. Journ.* August, 1885) a peculiar bacillus which occurs in the tissues and juices of bees, and especially their larvæ, which sometimes in beehives become affected with, and die of, the disease known as "foulbrood." This bacillus shows certain peculiarities in its mode of growth in nutritive gelatine and Agar-Agar, and is capable of forming spores. With such cultivations the disease was reproduced in healthy bees.

(*p*) *The Comma-Bacillus of Asiatic Cholera.*—Koch has discovered in the evacuations of persons affected with acute Asiatic cholera a peculiar vibrio, which he called comma-bacillus, and of which he states that it is intimately connected with the causation of the disease. Koch isolated these comma-bacilli from the mucus-flakes of the fluid contents

of the ileum by the method of plate-cultivation in nutritive gelatine described in Chapter V. The appearances presented by these comma-bacilli in such plate-cultivations are characteristic; they are shown in Fig. 95, but it is also shown there that a similar character is exhibited by some micrococci. These comma-bacilli multiply readily in the mucus-flakes taken from the lower part of the ileum of a person dead of acute cholera if these flakes are placed and kept on moist linen in a moist chamber.

The comma-bacilli grow and multiply well in neutral broth, neutral nutritive gelatine, neutral Agar-Agar mixture.

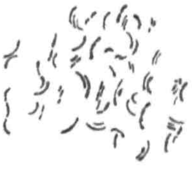


Fig. 96.—From a Preparation of Mucus Flakes of the Fluid in the Ileum of a Case of Typical Cholera.

Numbers of comma-bacilli of different lengths are shown amongst numbers of small straight bacilli. Magnifying power about 700.

An alkaline condition is not essential, as maintained by Koch, although, like other bacteria, notably putrefactive bacteria, they grow more luxuriantly if the medium is faintly alkaline. They grow well in milk, on potato (although the reaction of this latter is acid), and I have seen them grow even in faintly acid peptone broth.

The comma-bacillus of Koch is a curved rod of almost uniform thickness, sometimes slightly pointed at the extremities, its length about half that of a tubercle-bacillus, its thickness about the same as that of the latter. But the comma-bacilli vary in curvature and length within

considerable limits, some being just curved while others are almost semicircular, some being twice and three times as long as others. They are motile, and divide transversely. In neutral Agar-Agar mixture, kept at ordinary temperature, length-divisions may be observed (see Fig. 98). After transverse division they may remain joined end to end, and then forming an S-shaped corpuscle. But sometimes, particularly in artificial cultivations in broth, they remain joined end to end even after repeated division, and then form either a

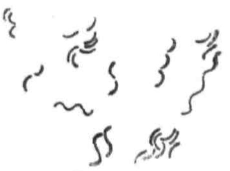


FIG. 97.

From an Artificial Cultivation of choleraic Comma-bacilli in Gelatine Peptone. Magnifying power 700. Most of these are single curved bacteria, a few are joined end to end in twos, thus forming S-shaped organisms; and a few are in chains of several placed end to end.

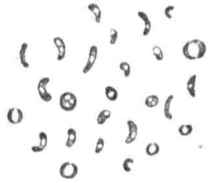


FIG. 98.

From an Artificial Cultivation of choleraic Comma-bacilli in Agar-Agar Peptone at the ordinary temperature of the room after several weeks. The Comma-bacilli change by vacuolation into planoconvex, then biconvex, and finally circular organisms; these by division give origin to two semicircular comma-bacilli. Magnifying power about 700.

wavy or spiral-like organism. But the type is represented by a single curved rod. For this reason it is not correct to speak of them as comma-bacill, since they correspond to what is generally considered a vibrio or spirillum.

The comma-bacilli are present, amongst crowds of other putrefactive bacteria, in very varying numbers in the choleraic evacuations, sometimes very scarce, sometimes numerous; in the mucus-flakes taken from the cavity of the lower part of the ileum of typical rapidly fatal cases of cholera very soon

after death they are present in small numbers, in the upper part of the ileum and in the jejunum they are either very scarce or altogether absent. The longer the examination is delayed, of course within certain limits, the more likely are the comma-bacilli to be found numerously in the flakes, but

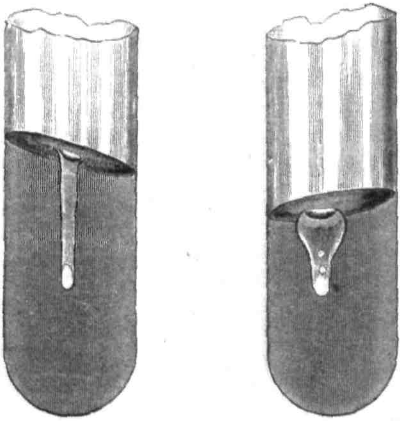
FIG. 99.

Part of a test-tube containing gelatine-peptone; in it pure cultivation of choleraic comma-bacilli. The funnel-shaped opening of the channel in which the growth of the comma-bacilli is going on contains a long air bubble.

not to the exclusion of other bacteria. They are generally absent from the mucous membrane itself, inclusive of the epithelium of the surface loosened but not detached. No organisms of any kind are found in the tissue of the intestine, in the blood, and other tissues; putrefactive bacteria,

including comma-bacilli, are capable of growing after death, and even before, into the clefts and spaces of the intestinal wall from the internal cavity.

The mucus-flakes of the small intestine, taken from a typical rapidly fatal case immediately after death, contain besides detached epithelial-cells, numbers of lymph corpuscles, some perfect, others swollen up and disintegrating.



FIGS. 100 AND 101.

Pure cultivation of choleraic comma-bacilli in gelatine-peptone-broth. The two tubes had been inoculated at the same time with the same comma-bacilli, and were kept under precisely the same conditions. In both the surface of growth is marked by a depression. At the bottom of the growth is a whitish precipitate of masses of comma-bacilli. The rest of the channel is filled with almost clear liquefied gelatine.

Soon after death all disintegrate. These corpuscles contain, in varying numbers within their protoplasm, straight minute bacilli much smaller than the comma-bacilli, being only half or a fourth their length, and more or less pointed.

But these bacilli are constantly found aiso free; singly, in chains, and in smaller or larger groups.

These small, straight bacilli are non-motile, and when grown artificially they form spores. Neither the comma-bacilli nor these small bacilli show in their mode of growth, in artificial cultivations in various media, greater peculiarities than other kinds of bacteria. The peculiar mode of growth of the comma-bacilli in gelatine mixtures is shown in the accompanying woodcuts.

FIG. 102.

Pure cultivation of choleraic comma-bacilli in gelatine-peptone-broth. The inoculation had been made, not in a channel, as in the previous figures, but on the surface. There is also here a depression of the growth on the surface, and in the extent of the growth the gelatine is liquefied, with a whitish precipitate at the bottom.

Both the comma-bacilli and small straight bacilli grow well in alkaline and neutral media, and are not killed by weak acids although they do not show growth in them, or only to a very limited degree. Neither the comma-bacilli nor the straight small bacilli can be considered as connected with the cause of cholera.

The result of the experiments performed by Nicati and

Rietsch, by Koch and others, viz., death following in some of the animals after injection of choleraic mucus-flakes or of cultivations of comma-bacilli into the cavity of the small intestine, were not due to cholera, but were probably due either to the operation or to septic poisoning. Rodents, carnivorous animals, and monkeys must be considered insusceptible to cholera.[1] There is direct evidence that the water contaminated with choleraic evacuations only, and of course with comma-bacilli, when drunk by a large number of persons did not produce cholera; there is no definite

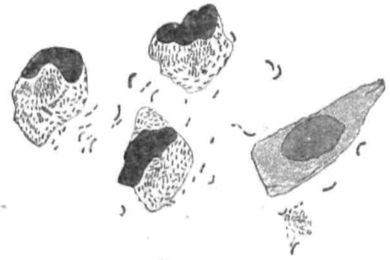


FIG. 103.

From a preparation of mucus flakes of the ileum of a case of typical cholera. Comma-bacilli and minute straight bacilli, singly and in masses. Three lymph-corpuscles containing in their interior numerous small straight bacilli.

evidence that a cholera patient elaborates ready virus and passes it out with the evacuations.

Comma-bacilli of various species have been discovered in other diseases of the alimentary canal; in the fluid of the

[1] In connection with all the experiments said to have yielded positive results on guinea-pigs, it must be mentioned that a variety of bacteria are known, which although derived from normal but putrid secretions, act very poisonously on rodents, *e.g.* the bacillus isolated by Bienstock from normal human fæcal matter; the dumb-bell micrococcus isolated by Pasteur and Sternberg from normal saliva, by Escherich from the stool of milk-fed infants, by myself from sputum of pneumonia.

mouth of normal persons (Lewis); in old cheese (Denike). The comma-bacilli found by Finkler and Prior in old stools of cholera nostras differ in size and mode of growth from Koch's comma-bacilli. So do those found in diarrhœa due to other causes. But some species in the fluids of the mouth are identical with Koch's comma-bacilli in their mode of growth.

The small straight bacilli above described are probably identical with those mentioned by Emmerich, and which are regarded by this observer as the true cause of cholera. On guinea-pigs they act very poisonously.

Koch has shown that if guinea-pigs be kept for twenty-four hours without food, then 5 cc. of a 5 per. cent. solution of sodic carbonate, and after twenty minutes, 10 cc. of a broth cultivation of choleraic comma-bacilli be injected into the stomach, and immediately after this 1 cc. of (German) tincture of opium for each 200 grammes weight of the animal is injected into the peritoneal cavity, the majority of these animals die after a day and a half to three days. The small and large intestine are found distended by, and filled with a clear fluid containing numerously the choleraic comma-bacilli. Experiments of this nature have no bearing whatever on the question of the causal relation of the comma-bacilli to cholera in man, for the following reasons:

(1) It is quite gratuitous to consider the disease from which those animals die as cholera, for during life they do not show any of the special symptoms characterising cholera.

(2) Koch himself has proved by numerous experiments that guinea-pigs treated in the above manner, but omitting the injection of opium tincture into the peritoneum, do not become ill at all, although even twenty hours after the introduction of the comma-bacilli, the stomach and small and large intestine contain them numerously in a living state, as is proved by the successful plate cultivations made by Koch himself from the contents of these organs.

(3) Death of the animals has been produced after precisely the same method by Finkler's comma-bacilli, and even by Denike's cheese comma-bacilli.

(4) Death does not ensue unless tincture—not watery solution—of opium be injected into the peritoneal cavity.

From all this it follows that the choleraic comma-bacilli are powerless to produce disease in guinea-pigs, even if present in the small and large intestine in a living state; *and that a previous pathological state of the* intestine, such for instance as is produced by the injection into the peritoneal cavity of considerable quantities of tincture of opium, enables the comma-bacilli to undergo multiplication.

CHAPTER XII.

VIBRIO.

VIBRIONES are characterised by being rod-shaped, but not straight; they are more or less wavy; and they are motile.

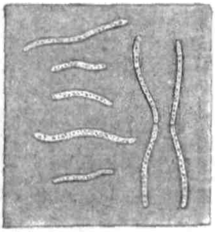


FIG. 104.—VIBRIO RUGULA (AFTER COHN).

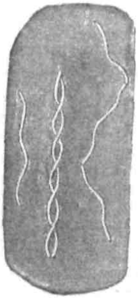


FIG. 105.—VIBRIO SERPENS, ISOLATED (AFTER COHN).

(*a*) *Vibrio rugula* consist of rods of about 0·008 to 0·016 mm. in length, and curved either like a C or like an S. They are single, or form chains of two. Their protoplasm is always slightly granular. They are found in putrefying organic substances, and often form continuous masses, the individuals interlacing in all directions.

(*b*) *Vibrio serpens*.—This is also a septic organism, much thinner and longer than the previous one, more wavy, as a rule, curved into a single or double wave. The length

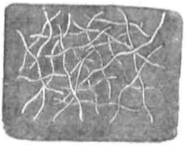


FIG. 106—VIBRIO SERPENS IN SWARMS (AFTER COHN).

varies between 0·011 and 0·025 mm. It is motile ; and also forms continuous masses, the individuals interlacing in all directions.

NOTE.—Many of the longer pathogenic bacilli, as bacillus of anthrax, of symptomatic charbon, of tubercle, of ulcerative stomatitis, &c., occasionally present themselves in forms closely resembling vibriones.

CHAPTER XIII.

SPIROBACTERIUM (*Spirillum*).

SPIRILLA are filaments of a spiral shape, motile, and owing to their shape follow a spiral course when moving. They are probably capable of forming minute bright spores.

FIG. 107.—SPIRILLUM TENUE, (1) SINGLY AND (2) IN SWARMS (AFTER COHN).

1. *Septic spirilla.*—These are found in all kinds of putrefying organic substances, and are of three kinds.

(*a*) *Spirillum tenue.*—This is much finer and more wavy than vibrio serpens, the turns being closer together and spiral. Its length varies between 0·002 and 0·005 mm.; it often forms continuous felted masses; it is motile.

Occasionally the spirilla grow to a great length—two, three, and more of them forming a chain; the individual spirilla are not arranged in a linear series, but folded into a zigzag. This form, which in reality is not a special kind of spirillum, is called by Cohn [1] *spirochæta plicatilis*. The spirillum found in the tartar of the teeth is of this form, *spirochæta denticola*. But there exist all intermediate forms between a single

FIG. 108.—SPIRILLUM UNDULA (AFTER COHN).

FIG. 109.—SPIRILLUM VOLUTANS (AFTER COHN).

spirillum tenue and a spirochæta. In stained specimens the construction of the spirochæta from several spirilla tenua is very distinct.

(*b*) *Spirillum undula* is much thicker and shorter than the former; there are all forms between such as are only

[1] *Beiträge zur Biologie d. Pflanzen*, vol. ii.

half a turn to such as are of a whole turn of a spiral. It is motile and forms chains of two or more elements, occurring also in continuous masses, occasionally held together by a hyaline interstitial substance.

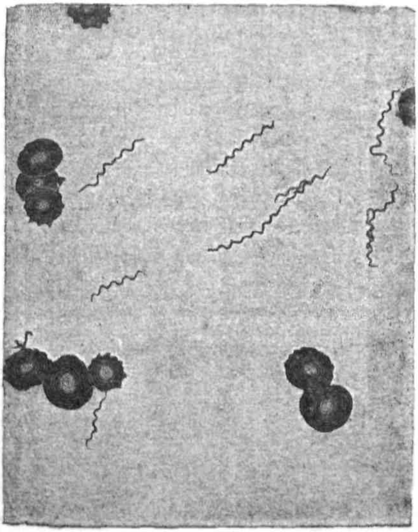


FIG. 110.—BLOOD OF RELAPSING FEVER (HUMAN). Blood-corpuscles and spirilla Obermeyeri. Magnifying power 700. (After Koch.)

(*c*) *Spirillum volutans.*—These organisms are giant spirilla; long and thick, with granular protoplasm; 0·025 to 0·03 mm. long; motile, and with a flagellum at each end.

2. *Pigment spirilla.*

(*a*) I have seen on paste a spirillum, morphologically identical with spirillum undula; it is of a pale pink

or rosy colour.[1] It is motile, and forms a kind of zooglœa, the individuals being closely placed and therefore producing a rosy colour of a more decided tint. Where they form continuous masses, the naked eye can detect the rosy tint.

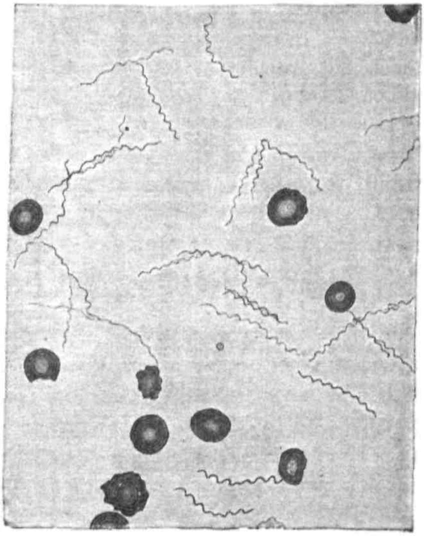


FIG. 111.—BLOOD OF APE INOCULATED WITH BLOOD SHOWN IN PRECEDING FIGURE.

Blood-corpuscles and spirilla.
Magnifying power 700. (After Koch.)

(*b*) *Spirillum sanguineum* (*Ophidomonas sanguinea* Ehrenberg).—This was observed by Cohn and Warming[2] in pondwater. Morphologically it is identical with spirillum volutans. It is motile, with a flagellum either at one

[1] "On a Rose-coloured Spirillum," *Quar. Journ. of Micr. Sci.*, vol. xv. New Series.
[2] *Beitr. z. Biol. d. Pflanzen*, vol. i.

or both ends. Warming occasionally saw two and three flagella at one end. It is about 0·003 mm. thick; all forms occur between such as have half and such as have two and a half turns of a spiral. Lankester also saw the same kind of organism amongst his peach-coloured bacteria.[1]

3. *Pathogenic spirilla.*

Spirillum Obermeyeri (of relapsing fever) is morphologically identical with spirillum tenue (or spirochæta plicatilis of Cohn). It was discovered in great numbers by Obermeyer[2] in the blood of the general circulation in patients suffering from relapsing fever. The spirilla disappear from the blood during the non-febrile stages, gradually decreasing in numbers. They are motile; they come out well in specimens of blood made after the Weigert-Koch method of drying the blood in a very thin layer, and then staining with methyl-violet or Bismarck-brown.[3] H. V. Carter[4] succeeded in producing relapsing fever in monkeys by inoculation with human blood containing the spirillum Obermeyeri. The blood in the monkey contained the same spirilla in great numbers. Koch has cultivated artificially the spirilla Obermeyeri, and saw them growing into long spiral threads.[5]

[1] *Quarterly Journ. of Micr. Science*, vol. xiii. New Series.
[2] *Centralb. f. med. Wiss.* 10, 1873.
[3] Weigert, *Deutsche med. Woch.* 1876; Heydenreich, Berlin, 1877.
[4] *Lancet*, 1879, vol. i. p. 84; and 1880, vol. i. p. 662.
[5] *Deutsche med. Woch.* 19, 1879.

CHAPTER XIV.

YEAST FUNGI: TORULACEÆ, SACCHAROMYCES.

YEAST, *torula* (Pasteur), or *saccharomyces*, is not a bacterium, but belongs to an altogether different order of fungi—the *Blastomycetes*. It consists of spherical or oval cells, very much larger than the largest micrococci, and as in the case of these each cell consists of a membrane and contents. The contents are either homogeneous or finely granular protoplasm; in the latter case there are generally present one, two, or more small vacuoles.

There are a great many species of *Torula*, varying from one another morphologically chiefly in their size, and physiologically by their action on various fluids (see below).

The cells multiply in suitable media by gemmation, a minute knob-like projection appearing at one side of the cell, and enlarging till it reaches nearly the size of the original or mother-cell. It finally becomes altogether constricted off from this latter, or having reached its full size remains fixed to the mother-cell, and each cell again producing by gemmation a new cell. In this way aggregations of four, six, eight, or more cells are formed, which may be arranged either as a chain when the production proceeds

in a linear manner, or as a group if the gemmation takes place laterally.

Under varying conditions of growth, *e.g.* on transplanting ordinary yeast growing in sugar-containing fluids on to potato, but sometimes also in the same nutritive fluid, it is observed that some of the yeast cells enlarge twice, thrice, and more times; they then form in their interior two, three, or more small cells by endogenous formation; these new cells are

FIG. 112.—TORULA, OR SACCHAROMYCES.

In the lower part of the figure an ascospore and four isolated spores (after Rees) are shown.
Magnifying power about 700.

regarded as spores[1]—the mother-cell being an *ascospore*—and become free by finally bursting the membrane of the mother-cell. On sowing these new cells into sugar-containing fluids they multiply by the process of gemmation.

Classifying them according to physiological function there are various species of torula or saccharomyces. They all

[1] T. de Seynes, *Comptes Rendus*, 1866; Rees, *Bot. Zeitschr.* 1869; Hansen, *Carlsberg Laborat.* 1883.

have the power to split up sugar into alcohol and carbonic acid, but this power is not possessed by all to the same degree.

(*a*) *Saccharomyces cerevisiæ* (*torula cerevisiæ*). This is the ordinary yeast used in the production of beer. The individual full-grown cells vary in diameter from 0·008 to 0·01 mm.; they form beautiful long chains. They produce ascospores.

(*b*) *Saccharomyces vini* is very common in the air, and produces alcoholic fermentation of grape-juice; it is therefore the proper yeast of wine-production. Its cells are elliptical, slightly smaller than the former; it forms ascospores.

(*c*) *Saccharomyces pastorianus* is of various kinds (Hansen): in some the cells are about 0·002 to 0·005 mm. in diameter, in others larger. Some form ascospores, others do not. Most of them can be found in wine-fermentation and in cider-fermentation, but only after the first alcoholic fermentation is completed. They are very common in the air. I have sown a saccharomyces, which was contained in ordinary water, on solid nourishing media (gelatine, and gelatine and broth). It grew up copiously and formed groups of a distinct pink colour. When growing in the depth of the nourishing medium it grew as a colourless torula, no ascospores were formed, multiplication taking place by gemmation only.[1]

(*d*) *Saccharomyces mycoderma* (*mycoderma vini*). This yeast is found forming the scum or pellicle on the surface of wine, beer, and fermented cabbage (*Sauerkraut*); its cells are oval, about 0·006 mm. long and 0·002 broad. It forms chains; the ascospores are two or three times larger. It has nothing to do with the alcoholic fermentation, and is not to

[1] *Quart. Journ. of Micr. Science*, 1883.

be confounded with *mycoderma aceti*,[1] which is a bacterium and the efficient cause of acid fermentation in wine and beer.

The saccharomyces mycoderma does not grow well in the depth of liquids, but when sown into a liquid of acid reaction and containing but little sugar, Cienkowsky saw the

FIG. 113.—SACCHAROMYCES MYCODERMA, OR OIDIUM ALBICANS. (After Grawitz.)

From an artificial cultivation in dilute nourishing material.

d. Branched mycelium.
f α. Torula stage.
f β. Mycelial stage.

cells elongating into cylindrical elements; each of which by gemmation produced a new cell which also elongated, and so on till a linear series of cylindrical cells was formed, separated from one another only by a thin septum; a mass of filaments very much resembling a mycelium was thus

[1] Nägeli, see chapter viii. 2.

formed. The cylindrical cells give origin by gemmation to spherical and elliptical torula-cells.

Such a growth, in which the torula-cells are capable of forming a sort of mycelium, was formerly called *oïdium*, and as *oïdium albicans* is recognised as the cause of "thrush;" the well-known white patches which occur on the mucous membrane of the mouth and pharynx in suckling infants and debilitated patients.

Grawitz[1] has proved by observations on artificial cultures that this fungus is identical with the oïdium variety of *Saccharomyces mycoderma;* the cells are spherical or cylindrical, the former about 0·003 to 0·005 mm. in diameter, the latter up to 0·03 or 0·05 mm. long. As above described it forms mycelium-like filaments from which, by lateral and terminal gemmation, spring spherical or oval torula-cells. It also forms ascospores containing four to eight spores.

[1] *Virchow's Archiv*, vol. lxx.

CHAPTER XV.

MOULD-FUNGI: HYPHOMYCETES OR MYCELIAL FUNGI.

OF this class of fungi only those are of special interest to the pathologist which in some way or other are connected with disease. The fungi consist of branched and septate threads or hyphæ; each filament or hypha is composed of a row of cylindrical cells, consisting of a membrane and clear protoplasm, the individual cells being separated from one another by a thin transverse septum; they increase in number by fission, and in this way the filaments increase in length. The growing ends of the hyphæ are filled, not with transparent, but with highly-refractive protoplasm. Some cells, by budding out laterally, produce cylindrical threads, which subdivide into a series of cylindrical cells, these by division and lengthening forming a new branch-hypha. The filaments form by their branches an interlacing feltwork, called thallus or mycelium. The mycelial fungi which interest us, belong to the order known to botanists as the *Ascomycetes*. They are characterised by the fact that one or other branch of the mycelial-hyphæ produces at its end a series of spherical or oval cells—the conidia-spores or *conidia*. In addition to this some of the hyphæ form peculiar large mother-cells, or *sporangia*, in the interior of which

spores are formed by endogenous formation. When these sporangia are cylindrical or club-shaped, they include eight spores, and are called *asci*; the spores being *ascospores*. All conidia or spores by germination grow into the mycelial threads which become septate or subdivided into a row of cylindrical cells; these by division cause the lengthening of the mycelial threads.

FIG. 114.—OÏDIUM LACTIS.
Mycelium and spores.

(*a*) *Oïdium lactis.*—Here the mycelium is composed of septate branched filaments of various thicknesses. Some branches of the mycelium at their ends or laterally at a septum produce by division a series of spherical or oval conidia-spores, about 0·007 to 0·01 mm. long. These ultimately become isolated, and then germinate into a short cylindrical

filament, which subdivides by transverse septa into a series of cylindrical cells; these by continued growth and division give origin to the ordinary septate branch-hyphæ. The formation of conidia proceeds at the ends of these in the same manner as before. The oïdium lactis forms a whitish mould on milk, bread, paste, potato, &c.

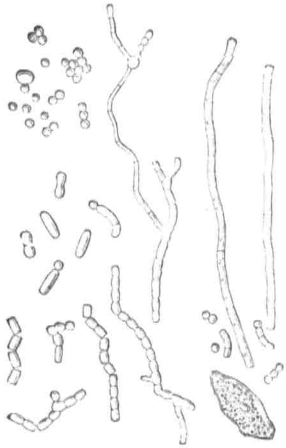


FIG. 115.—FUNGI FROM A FAVUS-PATCH (NEUMANN

Favus, Herpes tonsurans, and Pityriasis versicolor of man and animals, are, according to the researches of Grawitz,[1] due to a fungus in morphological respects identical with oïdium lactis. In favus it is known as *Achorion Schoenleini*, in Herpes tonsurans as *Trichophyton tonsurans*, in Pityriasis versicolor as *Microsporon furfur*. Grawitz has shown by artificial cultures on gelatine, that the spherical or oval

[1] *Virchow's Archiv*, vol. lxx. p. 560.

conidia germinate into shorter or longer cylindrical filaments, which by subdivision form septate mycelial hyphæ. These and their branches give origin in their turn to spherical or oval spores or conidia. They, as well as the hyphæ, differ in size in the various species.

Malcolm Morris and G. C. Henderson,[1] on the other hand, maintain, that by artificial cultivation of the spores of Trichophyton in the substance of gelatine-peptone, at temperatures varying from 15° to 25° C., these grow into branched septate mycelial filaments, which by their mode of fructification are seen to be identical with the mycelium of *Penicillium*. Compare also with Babes.[2]

(*b*) *Aspergillus*.—Some of the branches of the mycelium of this fungus assume an upright position, are thicker and not at all, or only slightly, septate, and at their end form flask-shaped enlargements, from which grow out radially short cylindrical cells—*basidia*; and these again at their distal or free ends produce chains of spherical spores or conidia. This is a very common mould, and according to differences in the coloration of the mycelium and spores is subdivided into different species; *A. glaucus*, *candidus*, *flavescens*, *fumigatus*, *&c.*

Besides this mode of spore-formation (asexual), there is another (sexual), which according to De Bary consists in this: some terminal branch of the mycelium becomes twisted like a spiral, this is considered the female organ of fructification or *carpogonium*; from the same thread branches grow towards the carpogonium; one of these branches becomes fused with the terminal portion of the carpogonium called the ascogonium, while the others—the *pollinodia*—branch and surround the carpogonium like a capsule: the

[1] *Journal of the Royal Microscopical Society*, April 11, 1883.
[2] *Archives de Physiologie*, 8, 1883, p. 466.

FIG. 116.—ASPERGILLUS GLAUCUS (AFTER DE BARY).
A. Hypha, the end of which, *c*, bears
st. The basidia.
as. Ascogonium.

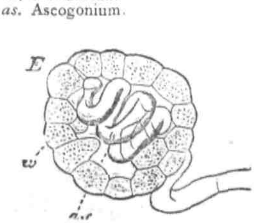


FIG. 117.—E. PERITHECIUM, HIGHLY MAGNIFIED
as. Ascogonium.
w. Cells of the pollinodia.

whole organ is now called a *perithecium*. Finally the ascogonium by rapid division gives origin to a number of oval septate tubes, inside of which by endogenous formation numerous spores make their appearance.

Grohe[1] was the first to show that the introduction of the spores of some species of aspergillus into the vascular system of rabbits sometimes produces death, with symptoms of metastasis into the various organs due to localised foci, where these spores grow into mycelial filaments. Lichtheim[2] showed that such mycoses in rabbits cannot be produced by the spores of *Aspergillus glaucus*, but by those of *Aspergillus flavescens* and *fumigatus*. Grawitz[3] studied this process more minutely, and found that, no matter whether the spores are injected into the vascular system or into the peritoneal cavity, there are established in the kidneys, liver, intestines, lungs, muscles, and occasionally in the spleen, marrowbones, lymphatic glands, nervous system, and skin, minute metastatic foci, due to the growth of the spores into mycelial filaments with imperfect organs of fructification, but no spore-formation. Grawitz thought that the spores of ordinary moulds (penicillium and aspergillus) are capable of assuming these pathogenic properties if cultivated at higher temperatures (39° to 40° C.), and in alkaline media. These fungi, as is well known, grow well at ordinary temperatures and in acid media, and are then innocuous when introduced into the animal body; but by gradual acclimatisation they can also be made to grow at higher temperatures and in alkaline media, when they assume pathogenic properties, becoming capable of resisting the action of living tissues and of growing in them. This view has been proved to be

[1] *Berl. klin. Woch.* 1871.
[2] *Ibid.* 9 and 10, 1882.
[3] *Virchow's Archiv*, vol. lxxxi. p. 355.

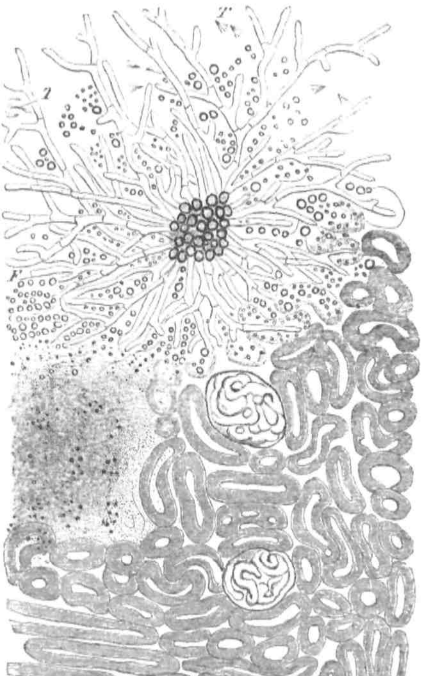


FIG. 118.—FROM A SECTION THROUGH THE KIDNEY OF A RABBIT DEAD THIRTY-SIX HOURS AFTER THE INJECTION OF SPORES INTO THE JUGULAR VEIN.

F. Fat droplets. *T*. Tyrosin crystals.

In the upper part of the figure is a metastatic focus composed of Aspergillus spores and mycelium. In the lower half of the figure the urinary tubules and two Malpighian corpuscles are seen. (After Grawitz.)

incorrect by Gaffky,[1] Koch,[2] and Leber.[3] Those spores that do exert such pathogenic properties are not at all

[1] *Mittheil. a. d. kais. Gesundheitsamte*, 1880.
[2] *Berl. klin. Woch.* 1881. [3] *Ibid.* 1882.

dependent on such acclimatisation, and are not ordinary moulds, but a distinct species of aspergillus (Lichtheim), which grows well at higher temperatures (38° to 48° C.), and the spores of which under all conditions of growth are capable of producing in rabbits the mycosis in question.

(*c*) *Penicillium.*—In this fungus hyphæ, which are not septate, grow out from the mycelium; from the end of each of these arise like the fingers of the hand a number of short branched cylindrical cells, which give origin to chains of spherical spores.

The following two fungi belong to the order of fungi called *Phycomycetes.*

(*d*) *Mucor* is characterised by this, that from the mycelium hyphæ grow out which are not septate, and at the end of these a large spherical cell originates, *sporangium*, in which by endogenous formation a large number of spherical spores are developed; the wall of the sporangium giving way, the spores become free.

(*e*) *Saprolegnia;* colourless tubular threads, forming gelatinous masses on living and dead animal and vegetable matter in fresh water. The cylindrical or flask-shaped ends of the threads—*zoosporangia*—form in their interior numbers of spherical or oval spores—*zoospores*, possessed of locomotion (one flagellum at each pole) and which finally escape from the threads. These zoospores after some time become resting, surround themselves with a membrane, and finally germinate into a cylindrical mass which becomes transformed into the mycelium. Besides this asexual there is, however, a second or sexual mode of fructification, consisting in this: At the end of a mycelial thread a cell grows up into a spherical large ball, the *oogonium*. From the same thread, thin threads—*antheridia*—grow towards the oogonium, with the protoplasm of which they merge. This latter then

differentiates into a number of spherical masses, the *oospores*, which become invested with a membrane. These become free and then germinate and grow into a mycelium.

FIG. 119.—SAPROLEGNIA OF SALMON DISEASE.
A sporangium filled with zoospores; in connection with them several young mycelial threads.

Saprolegnia grows on the skin of living fish, and causes here severe illness often terminating in death. Thus the

salmon disease, as Professor Huxley has shown,[1] is caused by this parasite. The zoospores of this salmon saprolegnia are, however, as Huxley has shown, as a rule non-motile. The hyphæ of the fungus traverse the epidermis in the diseased patches of the salmon, and they bore through the superficial layer of the derma, a stem-part being situated in the epidermis, and a root-part in the derma; each of these elongates and branches out. "The free ends of the stem-hyphæ rise above the surface of the epidermis and become converted into zoosporangia, more or fewer of the spores of which attach themselves to the surrounding epidermis and repeat the process of penetration." In saprolegnia associated with the salmon-disease Professor Huxley observed only the asexual mode of fructification.

An important case of general "*mycosis mucorina*" in man, ending in death, has been recently described by Dr. Paltauf (*Virchow's Archiv*, vol. 102, 3, p. 543). From the alimentary canal of the patient an invasion of the internal organs by the mycelium and spores of a kind of mucor occurred, leading to the formation of metastatic inflammatory foci in the Peyer's glands, lungs, pharynx, larynx, cerebrum, and cerebellum. In all these organs were found smaller and larger foci of inflammation caused by the presence of non-septate, branched mycelial threads and of sporangia, showing that the fungus belonged to the group of mucor.

[1] *Proceedings of the Royal Society*, No. 219, 1882.

CHAPTER XVI.

ACTINOMYCES.

In cattle there occurs a fatal disease, which is characterised by the formation of firm nodules of various sizes, due to a growth of small cells. In the centre of the nodules lie dense groups of peculiar club-shaped corpuscles—*actinomyces*—radiating from a firm homogeneous centre, and joined to this by longer or shorter, single or branched, filamentous stalks. Each of these actinomyces-corpuscles appears homogeneous, and of a bright slightly greenish lustre. These masses consist of what is called *Actinomyces* (Bollinger), and the disease is termed actinomycosis. In cattle the disease manifests itself by firm tumours in the jaw, in the alveoli of the teeth, and particularly by a great enlargement and induration of the tongue—"*wooden tongue.*" On making sections through this latter organ there are found present in all parts microscopic tumours of small-cell growth. In the centre of each tumour is a clump of actinomyces. This clump is surrounded by a zone of largish cells, with one to four nuclei. The periphery of the tumour is made up of a fibrous capsule, with spindle-shaped cells. Occasionally the tumours are to be seen also in the skin and in the lung; in the latter organ they appear as whitish nodules, easily

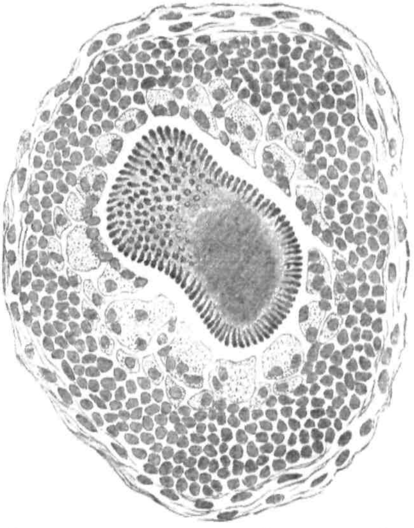


FIG. 120.—FROM A SECTION THROUGH THE TONGUE OF A COW DEAD OF ACTINOMYCOSIS.

A nodule is shown composed of round cells, in the centre is the clump of actinomyces surrounded by large transparent cells. Magnifying power 350.

FIG. 121.—A CLUMP OF ACTINOMYCES MORE HIGHLY MAGNIFIED, 700.

mistaken for tubercles.[1] Bollinger first described the disease in cattle.[2] Israel[3] was the first to point out a

[1] Pflug, *Centralbl. f. med. Wiss.* 14, 1882; Hink, *ibid.* 46, 1882.
[2] *Ibid.* 27, 1877.
[3] *Virchow's Archiv*, vols. lxxiv. and lxxviii.

disease in man characterised by metastatic abscesses (spreading it seems from a primary abscess of the jaw) in various internal organs, due to the presence of a fungus, which afterwards was identified as actinomyces, and Ponfick [1] has clearly established that in man it is not a rare disease.

According to careful observations, Johne [2] succeeded in transmitting the disease from cattle to cattle by inoculation, but not by feeding. He also found [3] in twenty out of twenty-one healthy pigs examined, the actinomyces present in the crypts of the tonsils.

Israel [4] succeeded in transmitting the disease to a rabbit by inserting into the peritoneal cavity a piece of a human antinomyces-tumour.

R. Virchow quite recently,[5] in conjunction with O. Israel and Duncker, ascertained that pork occasionally contained whitish chalky nodules, larger than those due to trichinæ, and containing in their interior the actinomyces.

O. Israel [6] claims to have succeeded in artificially cultivating the actinomyces on solid ox-serum; in fluid media the growth does not succeed, owing to the swelling up and death of the actinomyces-corpuscles.

[1] *Die Actinomykose des Menschen*, Berlin, 1881.
[2] *Deutsche Zeitschr. f. Thiermedicin*, vii. 1881.
[3] *Centralbl. f. med. Wiss.* 15, 1881.
[4] *Ibid.* xxvii. 1883.
[5] *Virchow's Archiv*, vol. xcv. p. 544.
[6] *Ibid.* vol. xcv. p. 142.

CHAPTER XVII.

ON RELATIONS OF SEPTIC TO PATHOGENIC ORGANISMS.[1]

THERE is hardly any question which to the pathologist and sanitary officer can be of greater importance than the relation of septic to pathogenic organisms. To the pathologist the life history of a micro-organism, outside and within the animal body, must ever remain an important field of inquiry; to the sanitary officer all conditions affecting the life and death of those organisms which produce, or at least are intimately bound up with, infectious diseases, such as the distribution and growth of these micro-organisms outside the animal body, the agencies which affect it in a favourable and unfavourable sense, are the points which he has particularly to consider in dealing with the spread and prevention of infectious maladies. Now, it is known of many micro-organisms, both those that are associated with putrefactive processes as well as those that are bound up with infectious disease, that temperature, the medium in which they grow, presence and absence of certain chemical compounds are capable of materially affecting them. I need not for this

[1] The greater part of this chapter is copied from an interim report by myself to the Medical Officer of the Local Government Board, 1884.

purpose enumerate all that is known already by direct experiment, but will only limit myself to reference to the researches of Schröter, Cohn, and Wernich on that group of micro-organisms known as pigment bacteria, *i.e.* bacteria which only under certain conditions, notably temperature and soil, produce definite pigments (Cohn's *Beiträge zur Biologie d. Pflanzen*); to those of Hansen (Carlsberg Laboratory) on yeast; to those of Neelsen on the bacilli producing the blue colour of milk, the bacillus syncyanus (*Beitr. zur Biol. d. Pflanzen*, iii. 2, p. 187); to the works of Toussaint, Pasteur, Chauveau, Koch, and others on the bacillus anthracis; Arloing, Thomas, and Cornevin on the bacillus of symptomatic charbon; of Koch on the bacillus of tuberculosis; of Israel on actinomyces, and many others; and particularly would I refer to the many valuable suggestions and considerations expressed by v. Nägeli in these respects in his book, *Die niederen Pilze*, München, 1877 and 1882.

While from these observations it would appear that both septic and pathogenic micro-organisms are capable of suffering some modifications in their morphological and physiological behaviour, it is nevertheless still an open question whether an organism which under ordinary conditions is only associated with putrefactive changes in dead organic material, and which cannot under these ordinary conditions grow and multiply within the living body, can, under certain extraordinary circumstances, become endowed with the power of growing and multiplying within the body of a living animal, creating there a pathological condition, inducing there an infectious disease.

Three distinct septic micro-organisms have, after numerous experiments and careful observations, been mentioned, as being capable when growing under certain extraordinary conditions of assuming pathogenic properties. These three

organisms are: (*A*) the common bacillus of hay infusion is said by Buchner to be capable of transformation into bacillus anthracis; (*B*) a bacillus subtilis, present in the air, which, although quite harmless in itself, assumes distinct pathogenic properties when growing in an infusion of the seeds of *Abrus precatorius*, becoming hereby endowed with the power of causing severe ophthalmia (Sattler); (*C*) a common mould, aspergillus, which harmless in itself, when grown on neutral and alkaline material at about body-temperature (38° C.) assumes, according to Grawitz, very poisonous properties, producing in rabbits inoculated with it death, with metastasis of aspergillus and its spores in the various internal organs.

There are in the literature of micro-organisms other cases mentioned, in which such a transformation has been *supposed*, but without any experimental proof, and we need not therefore trouble ourselves more about them.

Let us now review *seriatim* the above three cases:

(*A*) Dr. Hans Buchner in a paper, which for many reasons may be considered an important one, "Ueber d. experim. Erzeugung des Milzbrandcontagiums, &c.," published in the *Sitzungsberichte d. math. physik. Classe d. k. Bair. Akademie d. Wiss.* 1880, Heft iii. p. 369, states that he succeeded in transforming the common bacillus of hay infusion, the hay bacillus, into the bacillus anthracis.

The hay bacillus and the bacillus anthracis rank together morphologically under that form which Cohn has named bacillus subtilis.

The two are, however, not quite identical in morphological respects. The hay bacillus is a minute rod or cylindrical-shaped bacillus, which by elongation and division produces chains and further threads just like the bacillus anthracis, but in the latter (*i.e.* bacillus anthracis) the bacilli and their

threads are composed of cubical elements, as is shown in stained specimens, and as has been mentioned in a former chapter, whereas in those of the hay bacillus the elements are rods or cylinders. I have seen, however, many of the short hay bacilli which being constricted, *i.e.* in the act of division, appear as two short more or less cubical elements placed end to end. It is generally assumed that in hay bacillus the bacilli are always rounded at their ends, whereas the bacilli anthracis are as if straight cut at their ends; but this is not universally the case, since I have seen in cultures the bacilli anthracis with distinctly rounded ends. But, speaking generally, the hay bacillus is a rod more distinctly rounded at its ends, the bacillus anthracis of the blood is not so.

The bacillus anthracis is slightly thicker than the hay bacillus. In artificial cultivations carried on in neutral broth the bacillus anthracis is about twice as thick as the hay bacillus growing in the same fluid, and when both are growing in neutralised hay infusion the two are very conspicuously different from one another, and can at a glance be distinguished from one another; the hay bacillus being about half the thickness of the bacillus anthracis. In stained specimens, too, the latter is beautifully made up of a row of cubical cells, whereas the former consists of cylinders only.

True, the bacillus anthracis is not always of the same thickness, for, as I have shown, when growing in neutral pork broth it is decidedly thicker than in the blood of an animal dead of anthrax. And also in the blood of different animals the bacillus anthracis slightly varies in thickness, for in the guinea-pig's blood it is slightly thicker than in that of the rabbit or sheep.

The hay bacillus is motile, possessed of a flagellum, and

therefore capable of locomotion; the bacillus anthracis is not motile. I am quite aware that Cossar Ewart (*Quarterly Journal of Microscopical Science*, April 1878) states that he has seen in a specimen kept artificially heated under microscopic observation that the bacillus anthracis, at first non-motile, is capable of becoming motile. At one or both ends a flagellum grows out from its body. But this observation is unreliable, since Ewart did not guard himself in any way from the accidental introduction of septic bacilli, many of which are motile. Besides, he says of the bacilli, which he figures as anthrax bacilli, that they are connected with one another by two fine threads, and that they probably separate from one another and each retains one filament, which is its flagellum. But his observations, so far as they have application to anthrax bacilli, are capable of quite a different interpretation. In every specimen of blood and in every artificial culture bacilli can be seen, in which at one place or more the protoplasm is wanting, owing, as I have shown, to degeneration; in such places only the empty sheath is present and of course in fresh specimens this gives the appearance as if the two protoplasmic portions of the bacillus were connected with one another by two fine threads, *i.e.* the sheath being transparent is seen here edgeways.

In no instance has the bacillus anthracis been observed to be motile. I have examined thousands of specimens of fresh bacillus anthracis in the blood and in artificial cultures, and I have never seen anything that in the least would lead me to differ from this proposition.

As regards the spores they are of the same aspect and size in both the hay bacillus and bacillus anthracis. The threads in good cultures form in both cases the same bundles more or less twisted and forming convolutions, but in certain

cultures of the bacillus anthracis, *e.g.* in broth in which the growth is limited to the bottom of the fluid, the convolutions and the twisted condition of the threads are more pronounced, more cable-like.

Hay bacillus being motile, every culture of it is uniformly turbid, the bacilli being capable of moving about, and after a day or two of incubation at 35° C. they form a distinct pellicle on the surface of the fluid, which in further stages becomes complete and thick. These pellicles are composed of a dense feltwork of threads of the bacilli, and in them spore-formation is going on.

By shaking the fluid the pellicle sinks to the bottom, and if the fluid is not exhausted yet, a new pellicle is formed of the same nature.

In no culture of hay bacillus are there ever observed those cloudy, fluffy, whitish and transparent convolutions that are seen in cultivations of bacillus anthracis carried on at the bottom of fluid broth, and which have been so accurately described by Pasteur.

Both the hay bacillus and the anthrax bacillus when growing on gelatine mixtures liquefy the gelatine; both when growing in meat broth turn the at first colourless fluid in the course of incubation to an amber, and further to a brown, tint.

The hay bacillus is capable of thriving well in acid solutions, it grows copiously in hay infusion, which is of a distinct acid reaction; the bacillus anthracis, although capable of making a slight progress in acid hay infusion, does not get far, for degeneration soon sets in; it thrives best in neutral solutions. Hay bacillus thrives also very well in neutral solutions.

Buchner states, that by successive cultivation of bacillus anthracis under *constant variation of the nutritive material,*

he saw it assume gradually the properties of hay bacillus. Thus he saw that its mode of growth gradually changed, inasmuch as instead of forming, as the typical bacillus anthracis does, fluffy convolutions at the bottom of the fluid-nourishing medium, it gradually showed a tendency to stick to the glass and to the surface of the fluid, and to form a sort of pellicle just like the hay bacillus does. This I consider to be an erroneous interpretation of an easily explained and simple fact. It does not want any of the many successive generations of bacillus anthracis, in which Buchner says he has achieved this transformation; it simply requires two nourishing fluids in both of which the bacillus anthracis will thrive well, but which fluids differ in specific gravity. Let Buchner do as I have done, let him take two test-tubes, both containing sterile broth, but in one the broth concentrated, in the other dilute. Let him inoculate the two test-tubes with bacillus taken from the same blood, say of a guinea-pig dead of anthrax, let him place them in the incubator at a temperature of 35° to 38° C. After two or three days, and more decidedly later, he will notice this very difference in the aspect of the cultures that he lays so much stress on as indicating a change in the physiological character of the bacillus. One test-tube, containing the dilute broth, shows the typical fluffy convolutions at the bottom of the fluid; while the other, containing concentrated broth, shows a distinct attempt at the formation of a pellicle. Let him now take out a droplet from this second test-tube and inoculate with it two test-tubes of the same nature as above, *i.e.* one containing concentrated broth, the other dilute broth. After two or three or more days of incubation he will find exactly the same differences as above.

Buchner states that the bacillus anthracis when carried through a large number of successive cultures, at a tempera-

ture of 35° to 37° C., gradually loses its pathogenic properties. Of this assertion I have said already a great deal in my Report for 1881—1882, and I mention it here merely in connexion with Buchner's other assertions. I have shown, that even assuming that Buchner has had in all his cultures the true bacillus anthracis, but for which there is no definite proof, as Koch has so ably pointed out in his critical review of Buchner's work (*Mittheilungen aus dem k. Gesundheitsamte*, Berlin, 1881, Bnd. I.), Buchner having tested his cultures on white mice only, has fallen into a serious error, for, as I have shown (Reports for 1881—1882), a culture of bacillus anthracis may have become quite harmless to white mice, but be still very virulent to other animals. In fact, therefore, Buchner's result does not require for its achievement more than one culture, provided this has been kept for several days or weeks without spore-formation, as was the case in Buchner's experiments.

As regards Buchner's statement that by successive cultivation of bacillus anthracis at 35° to 37° C., this assumes the morphological and physiological characters of hay bacillus, I agree with Koch in regarding this as a complete error. If the cultures are quite safe from contamination nothing of the sort ever happens. I have now for several years carried on such cultures, and have not seen anything of the sort. It is of course clear that if by any accidental contamination, say at the time of inoculating a fresh tube, a motile septic non-pathogenic bacillus, with which, or with the spores of which, the air sometimes abounds, is introduced, every new culture established from this one will abound in this bacillus and as it grows quicker and more easily than the bacillus anthracis, the next cultivations become barren of all the bacillus anthracis, and only the non-pathogenic motile bacillus will be found present. This criticism has been

applied by Koch to Buchner's experiments, and I must fully endorse it.

But there is a much more serious statement of Buchner's —serious, because if true in nature, it is dreadful to contemplate to what amount of anthrax man and brute may become subject—viz., that he maintains to have succeeded in transforming the hay bacillus into bacillus anthracis, by carrying the former through many generations under ever varying change of soil. It is needless to detail here all these experiments of Buchner, since I do not attach any great value to them, and I should not have troubled myself much about them, were it not that one meets in mycological literature, particularly on the part of botanists, an acceptance of Buchner's statement that hay bacillus can change into the pathogenic bacillus anthracis (see Zopf, *Die Spaltpilze*, Breslau, 1883).

I have repeated Buchner's experiments on rabbits, guinea-pigs, and white mice. I have grown the hay bacillus in various kinds of broth, in gelatine broth mixtures, in hydrocele fluid, in peptone fluid, in Agar-Agar and peptone, at temperatures varying between 30° and 38° C., and I have, to put it shortly, never seen that it shows the least tendency to change its morphological characters, or that it ever assumes any morphological or physiological character like the bacillus anthracis. I consider this a perfectly hopeless task, and I feel sure any one might as soon attempt to transform the bulb of the common onion into the bulb of the poisonous colchicum.

But Buchner states that with his cultures of hay bacillus carried through many generations under varying conditions of soil, he inoculated white mice, which died under symptoms of anthrax, and whose blood contained the typical bacillus anthracis. I do not for a moment doubt that he really had

mice dying from anthrax after inoculation with cultures of hay bacillus, but I question the admissibility of his interpretation. I believe that some accidental contamination of the culture of hay bacillus with anthrax spores or otherwise may have occurred and have got overlooked. How liable one kind of infective material is to be invaded by foreign infective matter may be understood from the following examples of its actual occurrence.

It is now admitted on all hands that the results of Villemin in producing what is called artificial tuberculosis in guinea-pigs, by inoculating the animals subcutaneously with cheesy matter derived from human and bovine tuberculosis or from a guinea-pig suffering from artificial tuberculosis, cannot be produced by any other means; it cannot be produced by ordinary, *i.e.* non-tubercular cheesy or other pus,[1] nor by setons (as once thought by Wilson Fox and Sanderson) setting up chronic caseous inflammations in the skin of guinea-pigs, nor by chronic mechanical irritation, *e.g.* insertion into the peritoneal cavity of bits of gutta-percha or other substances producing chronic peritonitis (as was thought by Cohnheim and Fraenkel), but, as Cohnheim now tersely puts it, tuberculosis can be produced only by matter derived from a tuberculous source, and anything that produces this tuberculosis is derived from a tuberculous source. Dr. Wilson Fox, after the very important experiments performed by Dr. Dawson Williams, according to which chronic inflammation in the skin of guinea-pigs produced by setons, is in no case followed by tuberculosis, has conceded that in his earlier experiments there must have entered some error in the use of the materials. Cohnheim has conceded the same. It is clear that these observers, while working at the same period with both tuberculous and

[1] Compare Watson Cheyne, *Practitioner*, April 1883.

non-tuberculous matter, must have had, in the course of experiments with the latter substance, accidental contamination with the former, and hence had the guinea-pigs inoculated by them with non-tuberculous matter nevertheless affected with tuberculosis. Dr. Williams, who had no contamination to fear, working with non-tuberculous matter only, had consequently no accidental contamination. This shows us how dangerous, as regards reliability of results, it is to work in one laboratory with different infective materials at the same period.

I have myself experienced some very curious results bearing on this very point. During the last year I have seen the following cases of accidental contamination occur. I work in the laboratory of the Brown Institution, which comprises a suite of rooms. Although working extensively on anthrax, I generally limit myself to one room only. A friend of mine, who one day injected into a vein of a guinea-pig blood taken from a blood-vessel of a dog suffering from distemper, found, to his great disappointment, the guinea-pig dead after two days under the typical symptoms of anthrax, the blood of this animal teeming with the characteristic bacilli. The hypodermic syringe used in this experiment for injection had not been previously used by me in my anthrax experiments, since I never use a syringe in my inoculations, but only glass pipettes freshly made and drawn out into a fine tube. The experiment was performed in the room adjoining the one in which my anthrax investigations were being carried on, but I was in the habit of making every day a good many specimens of anthrax cultivations and spores, so that there must have been a good many of these spores distributed on the table and floor, and probably found their way into the wound of the guinea-pig at the time the above experiment was made.

Another gentleman working in the laboratory of the Brown Institution intended to inoculate several guinea-pigs with human tubercles. For this end he mashed up in saline solution, in a clean mortar, a bit of human lung studded with tubercles. He did this in my room on the same table on which I was working with anthrax. One of these guinea-pigs, inoculated with human tubercle, died before the second day was over of typical anthrax. Its blood was teeming with the bacillus anthracis. Such an accidental anthrax of a guinea-pig inoculated with tuberculous matter occurred several times. In all cases freshly drawn-out glass capillary pipettes had been used for performing the inoculation, and also the other instruments had been carefully cleaned before the inoculation.

I myself had the following accidental contaminations:—

A guinea-pig had been inoculated with a culture of bacillus anthracis, which I did not expect would produce anthrax, the culture not being capable of starting new cultures, the bacillar threads being all in a state of degeneration. The animal, of course, remained unaffected. Some weeks afterwards inspecting the guinea-pig, to my surprise, I found the inguinal lymphatic glands at the side of the former inoculation greatly swollen, filled with cheesy pus. The animal was killed, and was found to be affected with general tuberculosis, the cheesy matter of the tubercular deposits containing the tubercle bacilli. Comparing my notes on this animal with those of my friend Lingard, we found that on the very day on which I inoculated the animal with my anthrax culture we had inoculated several other guinea-pigs with tuberculous matter. This tuberculous matter was prepared in the same room in which I prepared the fluid for my anthrax inoculation, but the instruments in the two sets of experiments had not been the same.

A rabbit was inoculated with a culture of *bacillus anthracis* which I did not expect would produce anthrax. The animal remained unaffected with anthrax, but died after four weeks with the symptoms of extremely well-marked tuberculosis—in fact, the best marked case that I have seen—of both lungs, spleen, liver, and kidney. The tubercular deposits contained the tubercle bacilli.

Also in this instance inoculations with tuberculous matter had been going on at the same time, when I meant to have inoculated nothing else but a culture of anthrax bacilli.

I think all these facts taken together prove unmistakably that working with two contagia in the same laboratory and at the same period, accidental contamination is of no rare occurrence. And this applies with equal force to Buchner's experiments. Buchner worked extensively with anthrax cultures in the same laboratory, and at the same time he had those successful cases of anthrax in mice which he thought to have inoculated with cultures of hay *bacillus*, and accidental contamination probably was the result. Buchner himself has experimentally shown that anthrax virus in the shape of spores can by inhalation produce anthrax, and, therefore, this is another argument against his above cases of positive results. I am assuming that his cultures of hay bacillus were really free of spores of bacillus anthracis: but, seeing that his anthrax cultures were probably contaminated with hay bacillus, I do not see why, by some chance, one of his tubes which he thought he inoculated with hay bacillus should not have been accidentally contaminated with the spores of bacillus anthracis, of which there must have been many about in the air of the laboratory.

If Buchner could show us that in a laboratory, in which for some considerable time anthrax cultures, anthrax animals, and examinations of anthrax bacilli had not been

carried on, cultivation of hay bacillus ultimately yields a fluid which produces typical anthrax, then I should be perhaps prepared to concede his proposition of a transmutation of hay bacillus into bacillus anthracis. Such a proposition is of the widest importance, and therefore its proof ought to be beyond cavil, there ought to be no chance of a possibility of error. Such proof Buchner has not given, and I cannot therefore accept his interpretation.

(*B*) The second instance in which the transformation of a common septic into a specific or pathogenic organism has been experimentally achieved, or I should rather say has been stated to have been achieved, is the jequirity bacillus. In 1882 the well-known ophthalmologist M. L. de Wecker in Paris drew attention to the therapeutic value of the seeds or beans of *Abrus precatorius*, a leguminosa common in India and South America. The people of Brazil use it under the name jequirity as a means to cure trachoma, or granular lids. De Wecker after many experiments found that a few drops of an infusion made of these seeds causes severe conjunctivitis, in the course of which, no doubt, trachoma is brought to disappearance and cure, and it is accordingly on the Continent and in this country now used for this therapeutic object. [I am informed by my friend Dr. T. Lewis, formerly of India, now pathologist at the Netley Army Medical School, that the people in some parts of India know the poisonous properties of these seeds, and use it for inoculating with them subcutaneously, cattle ; in consequence a severe inflammation is set up, and the animals die of some sort of septicæmia. This is done for the sake of simply obtaining the hides of the beasts.]

Sattler, in a very important and extensive research (*Wiener medic. Wochenschrift*, N. 17-21, 1883, and *Klin. Monatsbl. f.*

Augenheilk. June 1883) ascertained that when an infusion of the jequirity seeds is made of the strength of about half per cent., this infusion after some hours to a few days contains numerous bacilli, motile, capable of forming spores, and in all respects identical with a bacillus subtilis. The bacilli are about 0·00058 mm. thick, and from 0·002 to 0·0045 mm. long. They form a pellicle on the surface of the infusion, and in the bacilli of this pellicle active spore formation is going on. The bacilli grow and multiply well at a temperature of about 35° C., but also, only slower, at ordinary temperature. Sattler cultivated artificially the bacilli on blood-serum gelatine and meat extract peptone gelatine, both solid media, and continued their growth through several successive cultivations. Both the infusions of the jequirity and the bacilli taken from these artificial cultures inoculated into the conjunctiva of healthy rabbits produce severe ophthalmia, leading to the production of great œdematous swelling of the conjunctiva and eyelids, and temporary closure of the latter, and to the secretion of purulent exudation. Both the exudation and the swollen lids are said to contain infective bacilli and their spores. Sattler ascertained by many experiments, that none of the bacilli and the spores distributed in the atmosphere had those specific properties, viz., to excite ophthalmia, as long as they grow in other than jequirity fluid, but having had access, *i.e.* having entered the jequirity infusion, assume here this specific power. There is no doubt that Sattler worked the whole problem with great care, worked out all points connected with it in great detail, and for this reason his work was considered to have for the first time unmistakably established that a harmless bacillus, owing to the particular soil in which it grew, assumes definite specific or pathogenic properties. To me this jequirity bacillus had a great interest, since I was

particularly anxious to get hold of such an organism, in order to see whether and how far it can again be made harmless. For if ever there was a good case, a case in which a previously harmless micro-organism had by some peculiar conditions become specific, this was a case; and therefore it must be here possible by altering its conditions of life again to transform it into a harmless being. The theoretical and practical importance of such a case must be evident to every one who has at all devoted any thought to the relation of micro-organisms with disease. The whole doctrine of the infectious diseases, I might almost say, is involved in such a case, for if in one case it can be unmistakably proved that a harmless bacterium can be transformed into a pathogenic organism, *i.e.* into a specific virus of an infectious malady, and if this again can under altered conditions resume its harmless property, then we should at once be relieved of searching for the initial cause in the outbreak of an epidemic. But in that case we should be forced to contemplate, as floating in the air, in the water, in the soil, everywhere, millions of bacteria which, owing to some peculiar unknown condition, are capable at once to start any kind of infectious disorder, say anthrax (Buchner), infectious ophthalmia (Sattler), and probably a host of other infectious diseases, and thus to form the starting-point of epidemics. And the only redeeming feature, if redeeming it can be called, in this calamity would be the thought that the particular bacterium would by and by, owing to some accidental new conditions, again become harmless.

These were the reasons, and good reasons I think they were, which prompted me to inquire into the jequirity bacillus and jequirity ophthalmia, and after a very careful and extensive series of experiments, to be described presently,

I have proved beyond any doubt that the jequirity bacillus, *per se*, has no more power to create an infectious ophthalmia than Buchner's hay bacillus had of creating anthrax.

The following experiments prove this conclusively :—

The seeds of jequirity (*Abrus precatorius*) are crushed and powdered, the perisperm is removed, and of the rest an infusion is made of about the strength of half per cent. with distilled water, previously boiled and contained in a flask previously sterilised (by heat) and plugged with sterile cotton-wool. The infusion is made while the water is still tepid. After half an hour the infusion is filtered into a fresh sterile flask, plugged with sterile cotton-wool, the access of air being limited as much as possible. This is effected by keeping the cotton-wool in the mouth of the flask around the end of the glass filter. The filtered fluid is of a slightly yellowish-green colour, and is almost neutral and limpid. A small quantity is withdrawn with a capillary glass pipette freshly drawn out, and from this several test-tubes containing sterile nourishing material (peptone solution, broth, Agar-Agar and peptone) are inoculated; and from the same pipette, and at the same time, several eyeballs of healthy rabbits are inoculated, by placing a drop or two of the infusion under the conjunctiva bulbi. The test-tubes are placed in the incubator and kept there at 35° C. After twenty-four hours all eyeballs are intensely inflamed, the eyelids closed and swollen, and a large amount of purulent secretion is present in the conjunctival sac, but all the test-tubes remain perfectly limpid; no growth has made its appearance, and they remain so.

In a second series the infusion prepared in the above manner is used fifteen minutes after it is made and used as above, for inoculation of test-tubes and eyeballs. The fluid in the test-tubes after incubation remains limpid, the eyeballs

all become inflamed. In both series the amount of fluid inoculated into the test-tubes is more than twice as great as that injected into the eyeballs. From this it is quite clear that the fluid used for inoculation of the test-tubes was barren of any micro-organisms, and nevertheless it possessed a powerful poisonous principle. I do not mean to say that the infusion as a whole contained in the flask contains no organisms, but that the small quantity of the fresh infusion that was used for the inoculation of the test-tubes and eyeballs contained none is absolutely certain. When such a flask is placed in the incubator, after twenty-four to forty-eight hours or later there are found in it large quantities of bacilli, the spores of which must have entered from the air during the process of preparing the infusion. The bacilli are such as described by Sattler; they soon form spores in the usual way. Such an infusion is very poisonous, just like the fresh one. Sattler has shown, and this is easily confirmed, that the spores of these bacilli stand boiling for a few minutes without losing their power to germinate. Consequently, if such a poisonous infusion full of bacilli and spores be boiled for half a minute the spores are not killed; proof for this: that if with a minute dose of this spore containing boiled infusion any suitable sterile nourishing material in test-tubes be inoculated, and then these test-tubes be placed in the incubator at 35° C., after twenty-four to forty-eight hours the nourishing fluids are found teeming with the jequirity bacilli; *but no amount of this material produces the least symptom of ophthalmia. Every infusion of jequirity loses its poisonous activity by boiling it a short time*, $\frac{1}{2}$ to 1 minute, and hence the above result.

In this respect the poisonous principle of jequirity infusion comports itself similar to the pepsin ferment, which, as is well known, is destroyed by short boiling.

If an infusion is made as above, and after fifteen minutes it is filtered and then subjected to boiling for $\frac{1}{2}$ to 1 minute, it will be found to have become absolutely non-poisonous, but not sterile : placing it in the incubator after twenty-four to forty-eight hours, vast numbers of the jequirity bacillus are found in it. But no amount of this fluid is capable of producing the slightest symptom of ophthalmia.

A large per-centage of the rabbits, whose conjunctiva has been inoculated with the fresh unboiled poisonous infusion, die after several, three to eight, days. The eyeballs and eyelids are intensely inflamed, as stated above, the skin and subcutaneous tissue of the face, neck, chest, and even abdomen, is found enormously œdematous, the pericardium, pleura, lungs, and peritoneum very much inflamed, their cavities filled with a large quantity of exudation. The exudations of the conjunctiva, pericardium, peritoneum, the œdematous skin and subcutaneous tissues contain no infective property, and as a rule no bacilli or spores of any kind, if examined in the living animal or immediately after death.

There is one point which requires careful consideration ; it is this : Sattler states that he has cultivated the bacillus, taken from a poisonous jequirity infusion, through several successive generations on solid material, and with the new cultures he was able to produce the jequirity ophthalmia. I have no doubt whatever that this is really the case, but it bears an interpretation different from the one Sattler gave it. Sattler, and most pathologists, would, of course, say this : if any micro-organism taken from a soil that possesses infective properties be carried through many successive artificial cultivations, all accidentally adhering matter would hereby become so diluted that it may be considered as practically lost ; that is to say, the organisms of the further

general mycosis, and this power they possess *ab initio*; other kinds of aspergillus do not possess this character and cannot acquire it under any conditions.

Thus also this third case of a transformation of a common into a specific organism due to altered conditions of growth falls to the ground.

It might be now asked, how about those cases in which by injection of very small quantities of putrid organic substances, pyæmia or septicæmia has been produced in rodents? Take the case of Davaine's septicæmia in rabbits. This disease has been produced in rabbits by Davaine, Coze and Feltz, and by many other observers, by injecting into the subcutaneous tissue of healthy rabbits small quantities of putrid ox's blood. The rabbits die in the course of a day or two, and their blood is found teeming with minute organisms, which prove to be bacterium termo; every drop of this blood possesses infective properties; when inoculated into a rabbit it produces septicæmia with precisely the same appearances as before. Pasteur and Koch have succeeded in producing septicæmia in mice and rabbits, and especially in guinea-pigs, by inoculating them subcutaneously with garden earth or with putrid fluid. This is Pasteur's septicæmia, or Koch's malignant œdema; it is characterised by œdema at the seat of inoculation, and spreading hence in the subcutaneous tissue of the surrounding parts. The animals die generally in twenty-four to seventy-two hours.

Koch has produced by injection of small quantities of putrid fluids into the subcutaneous tissue of mice a peculiar septicæmia; the animals sometimes die in forty to sixty hours, and the white corpuscles of the blood are found crowded with exceedingly minute bacilli. Koch succeeded also in producing a pyæmia in rabbits by injection of putrid fluids, and this pyæmia is characterised by zooglœa of minute

micrococci being present in the blood-vessels. Further, a progressive necrosis in mice by inoculating them with putrid fluids, the necrosis being due to the growth of micrococci and spreading from the seat of inoculation, and destroying as they spread all the elements of the tissue. All these cases have been minutely described by Koch in his classical work, *Die Aetiologie der Wundinfectionskrankheiten*, Leipzig, 1879. I have in addition mentioned in Chapter VII. § 13 a micrococcus causing abscess and pyæmia in mice.

Now do these cases prove that septic micro-organisms, living and thriving in putrid organic fluids, can, when introduced into the body of animals, owing to some peculiar unknown condition, so change as to produce a fatal infectious disease? I must say, with Koch, who has very ably discussed all these points, 'No.' Those organisms which are connected with the above morbid processes possess this pathogenic power *ab initio*, not due to any peculiar condition of growth.

Amongst the legion of different species of micrococci and bacilli occurring in putrid substances, the great majority are quite harmless; when introduced into the body of an animal they are unable to grow and to multiply, and therefore are unable to produce any disturbance. But some few species there are which, although ordinarily growing and thriving in putrid substances, possess this power, that when introduced into the body of a suitable animal they set up here a specific disease.

One of the best studied cases is that of the bacillus anthracis. This organism is capable of growing well and copiously outside the body of an animal, it thrives well wherever it finds the necessary conditions of temperature, moisture, and nitrogenous material; when it finds access to

the body of a suitable animal it produces the highly infectious fatal malady known as anthrax. The micrococcus of erysipelas is now well known through the admirable researches of Fehleisen to be capable of existence and multiplication outside the animal body; it grows well in artificial cultures, so does the tubercle bacillus of Koch, so does the bacillus which I described of swine-plague, mentioned in a former chapter, and so do other micro-organisms. Davaine's septicæmia in rabbits, Koch's septicæmia in mice, &c., &c., cannot be produced by every putrid blood or putrid organic fluid, only by some, only now and then, *i.e.*, when the particular micro-organism capable of inducing the disease is present in those substances, and then only when it finds access into a suitable animal. Davaine's septicæmia of rabbits cannot be induced in guinea-pigs, Koch's septicæmia of mice cannot be induced in guinea-pigs, anthrax bacilli cannot induce the disease in dogs, and so with the other micro-organisms.

We conclude then from this that some definite micro-organisms, although as a rule existing and growing in various substances of the outside world, have the power when finding access into the body of a suitable animal to grow and thrive here also, and to induce a definite pathological condition. But this power they have *ab initio*. Those that do not possess this power cannot acquire it by any means whatever. Just as there are species of plants which act as poisons to the animal body, and other species of plants which, although belonging to the same group and family, and although very much alike to the others, have no such power and cannot acquire such a power by any means, so there are micro-organisms which are pathogenic while others are quite harmless. The latter remain so no matter under what conditions and for how long they grow.

I have made a series of experiments with the view to obtain pure cultivations of definite septic micro-organisms: various species of micrococci, bacterium termo, and bacillus subtilis, of which the morphological characters could with precision be ascertained and which at starting were tested to be barren of any power of inducing disease. I have cultivated these in pure cultivations for many generations, and under varying conditions, and then I have inoculated with them a large number of animals (mice, rabbits, and guinea-pigs); and to put it briefly, I have not found that hereby any of them acquired the least pathogenic power.

CHAPTER XVIII.

VITAL PHENOMENA OF NON-PATHOGENIC ORGANISMS.

As has been stated in a former chapter, all putrefaction of organic matter is associated with micro-organisms. It is now generally admitted, because based on a large number of exact experiments (by Schwann, Mitscherlich, Helmholtz, Pasteur, Cohn, Burdon Sanderson, Lister, W. Roberts, Tyndall, and many others), that organic matter kept safe from becoming contaminated with micro-organisms of the air, water, and filth, remains free of them, and consequently of the form of decomposition which is generally considered as putrefactive; namely, the changing of proteids into soluble peptones; then the splitting up of these into leucin and tyrosin; further the decomposition of these and other crystallisable nitrogenous bodies into comparatively low compounds. These in their turn by oxidation ultimately yield ammonia, and its salts and nitrates of inorganic elements, with the simultaneous development of certain gases, *e.g.* ammonia, sulphurreted hydrogen, and other products, belonging to the aromatic series. The view now generally entertained is that the organisms cause disintegration of nitrogenous compounds by withdrawing from them certain molecules of nitrogen, building up with these their own pro-

toplasm. Similarly carbohydrates and inorganic salts, as phosphates, potassium, and sodium salts, are dissociated by them, inasmuch as they require a certain amount of carbon, phosphorus, potassium, and sodium, for building up their own bodies. In this process of decomposition certain alkaloids are produced, the composition of which is not accurately known, and which are called by the collective name of ptomaines (Selmi and others). These alkaloids are known to have a poisonous (toxic) effect when introduced in sufficient quantities into the system of a living animal. Very possibly the poisonous property of some articles of food, that have undergone putrefaction or some unknown kind of fermentation, is caused by some ferment, the product of micro-organisms; (sausage-poisoning, poisoning by bad fish and other articles). Brieger (*Die Ptomaine*, Berlin, 1885) has isolated from putrid proteid materials several alkaloids and has studied more accurately their toxic properties.

Gaspard, Panum, Bergmann, Billroth, Burdon Sanderson, and many others had already shown, that by putrefaction of animal substances, a substance can be obtained—the septic poison or sepsin—by various chemical processes which in themselves are destructive of every living micro-organism; this substance injected into the vascular system of animals, especially dogs, in sufficient quantities, produces a marked febrile rise of temperature, and is capable of causing death with the symptoms of acute poisoning, *e.g.* shivering, vomiting and purging, spasms, torpor, collapse and death. After death is found severe congestion and hæmorrhage of the lungs and intestine, particularly the duodenum and rectum; hæmorrhage in the pleura, pericardium, peritoneum, and endocardium. This *putrid infection*, identical with poisoning by ptomaines, leads to death in twelve to twenty-four hours, or even less. On injecting smaller quantities only a febrile

disturbance is noticed, severe symptoms and death only following after injection of considerable quantities, such as several cubic centimetres of putrid fluid. There is *a priori* no reason why something like putrid intoxication should not occur as a pyæmic infection in the human subject; if, for instance, at an extensive wound, *e.g.* after amputation of a limb, a large surface of suppurating tissue is established, on which, as is well known, numbers of putrefactive organisms are capable of growing, it is possible and quite probable, that here these organisms produce the septic poison, which when absorbed into the system in sufficient quantities produces septic intoxication. From this affection septicæmia proper, due to absorption of a specific organism by a small open wound or a vein, which increases within the body, and therefore is a living, growing, and self-multiplying, entity producing septicæmia, must be carefully distinguished.

These putrefactive processes must be distinguished from certain fermentative processes, in the course of which by introducing a definite micro-organism—zymogenic organism—into a definite substance, definite chemical products are produced. Thus the torula cervisiæ or saccharomyces introduced into a solution containing sugar, produces alcoholic fermentation, *i.e.* oxidation and splitting up of sugar into alcohol and carbonic acid.

The bacterium lactis introduced into substances containing lactic sugar, moist, or grape sugar, produces by oxidation a conversion of the sugar into lactic acid and carbonic acid (?). A micrococcus (see a former chapter) produces, according to Pasteur, the conversion of dextrose into a sort of gum, called by Béchamp viscose, and recognised by Pasteur as the cause of the viscous change of wine and beer. The urea in the urine is converted by the micrococcus ureæ (Pasteur) into

carbonate of ammonium. Solutions containing starch, dextrin, or sugar, infected with the bacillus amylobacter yield, as mentioned in a former chapter, butyric acid. The same bacillus converts glycerine (Fitz) into butyric acid, ethyl-alcohol, &c. Alcohol is oxidised by the presence of a definite micrococcus (Pasteur) into acetic acid.

A species of minute bacillus subtilis produces from fats butyric acid (Pasteur, Cohn), and many kinds of micro-organisms from pigmentary bodies, *e.g.* those producing the blue colour of milk.

What the chemical influence of pathogenic organisms on animal tissues may be is not yet known; and even when they grow outside the body, *i.e.* in artificial cultures, it is not yet known what their chemical effect on the nourishing material is, except that, as is the case with all other organisms, putrefactive and pathogenic, they continue to grow and multiply as long as there are present the necessary substances, *i.e.* until the medium is "exhausted."

From the enormous number of micro-organisms present in the outer world, it is clear that the *rôle* they play in the disintegration of higher organic bodies into lower compounds, as well as in the building up of new compounds, is a very important one; to mention but one series, to wit, the enormous importance they have for the vegetable kingdom in reducing nitrogenous compounds to soluble nitrates of inorganic salts, so essential to the existence and growth of our common field crops (Laws).

One of the most interesting facts observed in the growth of septic micro-organisms is this, that the products of the decomposition started and maintained by them have a most detrimental influence on themselves, inhibiting their power of multiplication, in fact, after a certain amount of these

products has accumulated, the organisms become arrested in their growth, and finally may be altogether killed. Thus the substances belonging to the aromatic series, indol, skatol, phenol, and others, which are produced in the course of putrefaction of proteids, have a most detrimental influence on the life of many micro-organisms, as has been shown by Wernich and others (see Antiseptics).

It is not well known whether in all or in some of these instances the organisms produce the chemical effects by creating a special zymogen or ferment, and by this create the chemical disturbance, or whether they merely dissociate the compounds by abstracting for their own use certain molecules; but this much is known, that in consequence of this chemical disturbance definite chemical substances are produced. It is quite possible that the pathogenic, like the zymogenic, organisms have this special character, that if the soil (animal body) contains a certain chemical substance, they are capable of growing and thus capable of producing a definite zymogen or ferment.

In many cases of putrefactive and zymogenic organisms a definite soil may be capable of furnishing suitable material for various organisms at the same time; as a matter of fact this is what one constantly meets with in ordinary putrefaction of vegetable and animal matter, which teem with various species of micro-organisms. But as a rule it will be observed that one species is more apt to find a suitable soil in this substance than others; and then it will be found that this one organism, above all others, grows more numerously; and when it has done growing, that is, when it has exhausted its own peculiar pabulum, another organism, not dependent on this, but on some other substance present, makes a good start and multiplies accordingly. Thus one finds constantly, that a fluid, supposing it contains a variety of proteids, carbo-

hydrates, and salts, having become infected with micrococcus, and various species of bacilli, in the first days or weeks chiefly micrococci are present; afterwards when the micrococci have done multiplying and sink to the bottom of the fluid, this latter gradually becomes filled with a variety of bacilli. Or if micrococcus and bacterium termo be sown at the same time in a suitable fluid, we find that at first only the bacterium termo increases rapidly; afterwards, when this has ceased multiplying, the micrococcus takes the field. In still other cases, as in putrid blood, exudation fluid, particularly in putrefying solid parenchymatous or other substances, various kinds of micro-organisms grow on simultaneously in different parts of the material.

The same holds good for the zymogenic organisms. A solution containing sugar is a very fit soil for saccharomyces; when this has exhausted the material, *i.e.* when all the sugar has been split up into alcohol and carbonic acid, the latter escaping as gas, then the material is ready for the organisms capable of converting the alcohol into acetic acid. The two kinds of organisms may be, however, growing at the same time, the saccharomyces leading.

Septic or putrefactive organisms then, like zymogenic and pathogenic organisms, are *cæteris paribus* dependent for their growth on the presence of the suitable nourishing material. And, as we have seen, they differ materially from one another in this respect; while putrefactive organisms find in almost all animal and vegetable fluids the substances necessary for nutrition, the zymogenic and pathogenic organisms are much more limited in these respects. Where there is no alcohol present the organisms producing the acetic acid fermentation cannot grow; where there is no sugar or a similar substance present the saccharomyces cannot grow, and so also a particular pathogenic organism—

the bacillus anthracis—cannot grow in the living tissues of the living pig, dog, or cat, but grows well in those of rodents, ruminants, and man; the bacillus of swine-plague grows well in the pig, rabbit, and mouse, but not in the guinea-pig or man.

Septic organisms differ also from pathogenic organisms in this, that the former are capable of growing in fluids containing only simple nitrogenous compounds, *e.g.* tartrate of ammonia, whereas pathogenic organisms require more complex combinations, proteids, or allied nitrogenous substances. Thus, for instance, in Cohn's and Pasteur's fluids septic micrococcus, bacterium, and bacillus grow well and copiously, but pathogenic organisms absolutely refuse to grow in them; even bacillus anthracis, which appears the least selective, cannot make a start in it. Some organisms, *e.g.* tubercle-bacilli, require the most complex nitrogenous compounds; they refuse to grow, for instance, in broth in which anthrax-bacilli, bacilli of swine-plague, micrococcus diphtheriticus and erysipelatous can grow well.

All septic and zymogenic organisms properly so-called, and described in former chapters, differ in this essential respect from pathogenic organisms, that the former two absolutely refuse to grow in the living tissues of a living animal.

As was stated in former chapters, it is not at all uncommon to find masses of micrococci in tissues which during the life of the subject have become dead or necrotic, or so severely changed by inflammation or otherwise that they may be considered as practically dead. In the diseased, necrotic intestine, the liver, the spleen, in abscesses, in the subcutaneous, submucous, or parenchymatous tissues, masses of micrococci have been noticed which in no way bear any intimate relation to disease, merely finding in the dead or

severely diseased tissues a suitable nidus for their growth and multiplication. But they may be present even in organs which show no severe disorganisation; thus, for instance, in fatal cases of small-pox, typhoid fever, pyæmia, even infantile diarrhœa, masses of micrococci may be found in and around the blood-vessels in the liver and spleen. In all these cases the micrococci are capable of growing, because owing to the severe general disorder these tissues have before the actual death of the patient lost their vitality, and, consequently, are unable to resist the immigration and settlement of the micrococci. Of the same character are the masses of bacilli one meets with sometimes in the intestinal wall, liver, and mesenteric glands after death from severe disorder of the bowels, *e.g.* typhoid fever and dysentery. I cannot for a moment accept the view of Klebs and Koch, that the presence of the bacilli mentioned in a former chapter necessarily stands in any causal relation to typhoid fever, seeing that they are not constant, and particularly that they are found in organs directly connected with the intestines, which we know are in this disease in an intense state of disorganisation.

The question arises: Where do the micrococci and bacilli come from which are thus capable of settling in a disorganised tissue even during the life of the subject? There can be no doubt that, as regards the intestinal wall, the mesenteric glands, the liver, and the spleen, the organisms could readily, in cases of severe disorganisation of the intestine, immigrate from the cavity of the bowel, where they are normally present, into the wall of the intestine, and moreover be absorbed together with the products of disorganised tissue into the mesenteric lymphatic glands, the liver, and the spleen. Further, it is not difficult to explain that if a focus of inflammation or necrosis be set up at

various internal places in consequence of emboli carried from an inflammatory focus to which micrococci or bacilli from the outer world have access, *e.g.* the skin, alimentary canal, respiratory organs, these internal places or metastases would harbour the same organisms, and as soon as disintegration—abscess, caseation, or necrosis—takes place in these metastases, also the imported organisms would multiply to a great extent, the tissue being shut out from the circulation and practically dead.

All this I say is not difficult of explanation if we bear in mind that the products of an inflammatory focus to which organisms have ready access from the outer world become themselves a ready nidus for these organisms; and when some of these products are absorbed and taken into the general circulation to act as emboli and thus to set up inflammation in distant regions, the organisms, embedded in and shielded by those products from any destructive action the living blood in the circulation may be capable of exerting, are thus transported to the new foci of inflammation and disintegration, resulting from the emboli. All this is self-evident, and does not require more proof than what is already known, and it follows from such considerations that the presence of micrococci and bacilli in the tissues of internal organs, in severe cases of disease, when some of the organs become disorganised before the actual death of the body, or in secondary foci of severe inflammation and necrosis, may have no connection whatever with the original cause of the disease or necrosis, but may be, and probably is, simply due to a transportation and immigration of non-pathogenic putrefactive organisms.

A most striking case of this kind I met with in mice dead of swine-plague; the bowels were severely inflamed, and in the liver there were present necrotic patches, an almost

constant symptom of the disorder; in such necrotic patches the capillary blood-vessels are sometimes, not always, found distended and plugged with the zooglœa of putrefactive micrococci, which have nothing to do, specifically, with the real disease (see a former chapter).

The cavity of the alimentary canal, small and large intestine, especially the latter, contains under normal conditions innumerable masses of putrefactive micro-organisms. These being much smaller than chyle-globules, must of necessity become as easily absorbed as the latter by the lacteals, and by these are carried into the general circulation; but being putrefactive they are unable to resist the action of the normal blood, and therefore in the healthy condition perish. But if there be in any part a focus of disorganisation they can settle there and propagate, provided they get there through the blood in a living condition. Many experiments prove that they cannot pass unscathed through the normal healthy blood, and therefore it is not probable that they would reach such a focus in a living state; but let them be well inclosed in a solid particle, say of disorganised tissue, and then carried through the vascular system, and we can quite understand that in this state, *i.e.* in and with that particle, they may reach the distant focus in a living state, and if in this focus the conditions are favourable for their growth, *e.g.* if there is inflammation and necrosis, we may expect them to multiply accordingly.

It is now admitted by most competent observers that in the healthy and normal state the blood and tissues contain no micro-organisms whatever, and that the assertions to the contrary are due to errors in the experiment, *i.e.* to accidental contamination. I will on this point merely refer, amongst many others, to the observations of Watson Cheyne [1] and

[1] *Pathological Transactions*, vol. xxx.

F. W. Zahn.[1] Consequently it cannot be maintained that if in any focus of disintegration micro-organisms make their appearance they are derived from those normally present; we must, on the contrary, assume that putrefactive organisms can be imported from parts connected with the outer world into distant localities in which disorganisation of tissues has taken place.

It is clear from the foregoing that after death micro-organisms will readily immigrate into the various tissues, and in this respect those organs situated near places where under all conditions micro-organisms exist will be the first to be invaded by them; *e.g.* the lungs, from micro-organisms present in the bronchi and air-cells derived from the outer air, the walls of the alimentary canal, the mesenteric glands, the peritoneal cavity, the liver and the spleen. The bacilli possessed of locomotion are particularly to be mentioned in this respect, but other non-motile bacilli and micrococci also find their way into these organs; thus Koch[2] saw only a few hours after death bacilli (non-motile) present in the blood of the arteries of a healthy person who had died by strangulation.

Quite recently Bizzozero has shown (*Centralbl. f. d. med. Wiss.* No. 45, 1885) that in perfectly normal and living rabbits the lymphatic tissue of the Peyer's glands of the intestine contains bacteria, either free between the lymph-cells, or aggregated in groups within the protoplasm of certain large lymph-cells. I have myself had opportunity to verify this statement. In a perfectly healthy rabbit immediately after killing it, the peritoneum and outer muscular coat is stripped off, then with a clean and sterilized scalpel a scraping is made from the lymphatic tissue of the deepest part of a Peyer's gland, of course care being taken that the mucous membrane lining the intestinal cavity remains unopened. With the scraping cover-glass specimens are then made,

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[1] *Virchow's Archiv*, vol. xcv.
[2] *Pathogene Micro-organismen.*

dried, stained with gentian-violet, and mounted. In such specimens large cells are found, the protoplasm of which is filled with beautiful small bacilli. When, therefore, speaking of the occurrence of bacilli or other bacteria in the tissue of the lymphatic glands or other parts of the wall of the bowels of a person dead of some disease or other, it is necessary to bear in mind the above facts, viz., that already under perfectly healthy conditions, even during life, bacteria can make their way from the internal cavity into the tissue of the intestinal wall.

Metschnikoff has pointed out (*Virchow's Archiv*, vol. 97, 3, p. 502) that amœboid cells in the blood and connective and lymphatic tissues are capable of embodying bacteria introduced into the tissues, and he called those cells phagocytes. While there is no doubt that bacteria like other granules and particles can be swallowed by amœboid cells, it is manifestly going too far to say, as Metschnikoff is inclined to do, that the phagocytes play an important part in neutralizing the action of pathogenic bacteria introduced into the blood and tissues by quickly swallowing and destroying those bacteria. Where in a tissue pathogenic bacteria find the suitable conditions for growth and multiplication, they can do successful battle against the amœboid cells, but where those conditions do not obtain, the bacteria linger and die, and like other particles can be swallowed up by the amœboid cells. That the presence of bacteria in the protoplasm of amœboid cells does not indicate that the former are being removed or destroyed and their action neutralized by the latter, but exactly the contrary, is proved in Koch's septicæmia of mice, in bovine tuberculosis, in leprosy, and in other diseases detailed in former pages (see Chapter XI.).

The assumption that where the leucocytes (phagocytes) are capable and sufficiently numerous to take up and destroy the pathogenic bacteria, no disease follows and immunity is the result, is contrary to some elementary facts, *e.g.* however small the number of anthrax bacilli or tubercle bacilli introduced into the blood of a susceptible animal, infection sets in with certainty, while no infection follows in an animal unsusceptible to the disease however large the dose of these bacilli. No one would be bold enough to say that the white blood-cells differ in the two animals in numbers and characters. Or to put it more strongly: it would be absurd to say that in a sheep which has passed through a mild form of anthrax, and as is well known has hereby become unsusceptible to a second attack, the leucocytes have altered in number and character, so that before the first attack they have been unable to swallow up and destroy the anthrax bacilli, but by the first attack had become endowed with this new power.

CHAPTER XIX.

VITAL PHENOMENA OF PATHOGENIC ORGANISMS.

As has been stated in the preceding chapter, the specific micro-organisms have the great differential character that they are capable of existing and propagating themselves in healthy living tissues. In those species in which the complete series of proofs has been furnished to establish the fact that the micro-organisms are intimately associated with the cause of the malady (*e.g.* malignant anthrax, tuberculosis, swine-plague, erysipelas), in which it has been shown beyond doubt: (*a*) that an animal suffering from the malady contains in definite distribution the particular micro-organism, (*b*) that the micro-organisms, cleared by successive artificial cultures from any adhering hypothetical chemical virus, when introduced into a suitable animal produce the malady, (*c*) that every such affected animal contains the micro-organism, in the same distribution and relation to the diseased organs as the original animal dead after disease—in those instances, I say, the only way of understanding the effect of the micro-organisms is to assume, what is actually the case, that the micro-organisms introduced into the living tissue go on multiplying, and directly or indirectly, *i.e.* themselves or by their products, as will be stated below, produce certain

definite disorders in the different parts. In the most favourable cases (anthrax, tuberculosis), a single organism introduced into a suitable locality in the animal body will be capable of starting readily a new brood. But in other cases it is necessary that an appreciable number of the organisms be introduced in order to start a brood. This was the case in some of the septicæmic processes in rodents studied by Koch.[1] The period between the time of introduction of the organism into the body (blood, skin, or mucous membranes, subcutaneous tissue, lungs, alimentary canal) and the production of the new brood large enough to produce a definite effect locally or generally, corresponds to the incubation-period of the disease, and, as is well known, there is in this respect a great difference in the different diseases. Thus in anthrax the introduction of the bacilli into the subcutaneous tissue of a suitable animal is followed after from sixteen to twenty-four hours or more by a local effect (œdematous swelling), and a few hours after by general constitutional illness, when bacilli can as a rule be found in the blood. On the other hand, in tuberculosis after the introduction of the *bacilli tuberculosis* into the subcutaneous tissue, the nearest lymph-glands show the first signs of swelling and inflammation after one week or even later, and the general disease of the internal viscera does not follow until one, two, or more weeks have elapsed. This is also borne out by observations of the behaviour of these bacilli in artificial cultures; whereas a suitable material inoculated with the bacillus anthracis and kept at the temperature of the animal body (38° to 39° C.), shows already after twenty-four hours a good crop of the bacilli; in the case of the tubercle-bacilli the first signs of a new brood are not noticed, as Koch has pointed out, and as I have had in several

[1] *Infectionskrankheiten, loc. cit.*

instances occasion to verify, before ten to fourteen days have passed.

One of the most important points, and the most difficult of comprehension, is this power of the pathogenic organisms to resist the influence of the healthy tissues of the living animals, a power which we said above is not possessed by the non-pathogenic organisms. A careful analysis shows at the outset that this power of the pathogenic organisms is not possessed by them indiscriminately, for while a particular species is in some animals capable of overcoming the influence of the living tissue, *i.e.* to multiply and to produce the particular disease, in other animals it is not capable of doing this, and hence the animal remains unaffected—it is said to be not susceptible to the disease. Thus, for instance, the bacillus anthracis when introduced into a human being or a herbivorous animal, is capable of multiplying and of producing anthrax, whereas in carnivorous animals and even in the omnivorous pig it is not capable of doing so. Or again, the bacillus of swine-plague while capable of producing the disease in swine, rabbits, and mice, is not capable of doing so in man, bird, the guinea-pig, or carnivorous animals. Now, where are we to look for this difference in behaviour? The tissues and juices of a pig when obtained as infusion or otherwise are just as good a nourishing material for the bacillus anthracis as the tissues and juices obtained from a herbivorous animal; artificial cultures of the former and of the latter behave in exactly the same manner, both as regards copiousness of growth and virulence of the bacilli. Again, artificial cultures of the bacillus of swine-plague made in juice of the tissues of the guinea-pig or fowl are exactly the same as those made of the juice of the tissues of a rabbit or pig. The tissues, therefore, *per se* cannot be said to possess any inimical action on the organisms. The living condition

per se can also not have this power, since we see that the power to overcome the influence of the living tissue is precisely the great distinguishing character of pathogenic organisms. There remains, therefore, only one thing, that is that there is something or other present in a particular tissue to which this latter owes its immunity, and this something must of necessity be connected with the tissue while alive, as we said before. Now, the life of the tissue in the pig cannot be different from that of the mouse, if by life is understood the function of the tissue, the connexion with the vascular and nervous system, and all the rest of it. The subcutaneous connective tissue has no different function, no different relation to the vascular and nervous system in the pig from what it has in the mouse, and nevertheless we find that it behaves so differently in the two cases towards the bacillus anthracis. This something then, which inhibits the growth and multiplication of the bacillus anthracis in the tissue of the pig but not in the mouse, must be something which, although dependent on the life of the tissue, is not identical with any of the characters constituting the life of the tissue, but must be some product of that life. To assume then, as is done by some observers, that the living state of the cells *per se* is the inhibitory power does not cover the facts, as we have just shown. The most feasible theory seems to me to be this, that this inhibitory power is due to the *presence of a chemical substance* produced by the living tissues. It does not require any great effort to conceive, and it does not seem at all improbable, that the blood and tissues of the pig contain certain chemical substances which are not present in the mouse, substances which like so many others chemistry is not yet capable of demonstrating. But that there exist vast and gross differences in the chemical constitution of the blood and tissues of different species

of animals there can be no reasonable doubt; it is a fact with which physiological chemistry is quite familiar.

We arrive then, after all this, at the conclusion that owing to the presence in the blood and tissues of particular chemical substances, present only during life, and a result of the life of the tissue, the organisms in a particular case cannot thrive and produce the disease. And further, that for each particular species of organism there is a particular chemical substance required to exert this inhibitory power, for, as we have seen, while the anthrax-bacillus is not capable of thriving in the pig, it does well in the guinea-pig, while the bacillus of swine-plague thrives well in the pig, it does not in the guinea-pig. The incapability of non-pathogenic organisms to thrive in healthy living tissues would on this theory be explained by the assumption that these chemical substances present in every healthy living tissue are inimical to all putrefactive organisms.

What we have said hitherto refers only to the healthy living tissues. It is quite possible to imagine that owing to the presence of a particular chemical substance in the healthy living tissue, a pathogenic organism is not able to thrive in a particular animal; but under certain abnormal conditions, when for instance owing to a diseased or altered state of the tissue that substance is absent, the organism might be enabled to exist and multiply and to produce the disease. Supposing it to be true that a person so long as he is healthy and well is able to successfully withstand the growth of a particular pathogenic organism, he is then unsusceptible to the disease; but we can understand that if the alimentary canal or the lungs be diseased, then the organism passing into the bowels by ingestion or into the lungs by inspiration would find there a tissue in which the necessary inhibitory substance, present in the healthy state, might be absent, and the organism

would be capable of thriving and of producing the disease.

The next point to consider is the relation of the specific micro-organism to the essence of the disease, or in other words the question whether the organism itself is the virus or whether this latter is the product of the former, something in the same way as the septic ferment is the product of the putrefactive organisms?

That the virus in the infectious diseases is intimately connected with the organisms is proved by the fact that the virus introduced into the body multiplies *ad infinitum;* for instance, in anthrax or tuberculosis after the introduction of an infinitesimal dose, we find the disease (malignant anthrax or general tuberculosis respectively) sets in in its virulent form; in the first case every drop of blood teems with the bacillus anthracis; in the second (tuberculosis) every tuberculous caseous particle in the lymph-glands, lungs, spleen and liver contains the bacilli; in both instances crops of the bacilli are produced in the afflicted body, and every particle of the tissue containing the bacilli is capable of starting the disease when introduced into a fresh subject. Moreover the artificial cultures of the pure bacilli are possessed of the same pathogenic power. The same holds good for leprosy, for erisypelas, for swine-plague, &c. So that the proposition that the organisms are intimately connected with the virus must be considered as well established.

But even after this it remains an open question whether the organism is identical with the virus, or whether the organism is concerned in elaborating the virus—a sort of ferment; and further, whether the virus being the latter's product, is obtainable apart from the organism.

Let us start with the proposition that the virus is a product

of the organism, a sort of non-organised ferment, but not the organism itself, although this latter is essential for the creation of the latter.

Inoculating a few bacilli anthracis into the subcutaneous tissue of a suitable animal, *e.g.* a guinea-pig, we find after twelve to twenty-four hours the first indications of illness, consisting in a local swelling and a general rise of the body-temperature. At this time there are present in the local swelling bacilli, but only in small numbers ; in the blood the bacilli are very scarce indeed, so scarce that it is difficult to meet with one bacillus in an appreciable quantity of blood. By this time then the bacilli could not have produced the change by their "numbers" alone. Immediately before death, sometimes some hours, we find in most instances the blood teeming with the bacilli, but this is by no means in all cases ; I have seen a considerable number of deaths from typical anthrax in the mouse, guinea-pig, rabbit, and sheep, occurring from forty-eight to sixty hours after inoculation, in which the number of bacilli of the blood and tissues was extremely small ; they were present, but only here and there was there one to be found. That the bacilli are present some hours before death in the shape of spores, as has been maintained by Archangelski, I have disproved in a former chapter. In those cases in which the bacilli are scarce even *in articulo mortis* and immediately after death, the scarcity is not due to the bacilli having already degenerated, since the degenerating bacilli are not noticeable in any way in these instances. It remains then to assume that death occurs in these cases not owing to the presence of the bacilli in numbers ; that is to say, that it is neither owing to their appropriating from the blood-corpuscles the available oxygen necessary for their multiplication (Bollinger) and thus producing death by asphyxia, nor to their mechanical effect in

plugging up the capillaries of vital organs, a theory upheld by some observers from the fact that in most cases the capillaries appear filled with the bacilli, and in some cases in extensive regions, lung, kidney, and spleen, the capillaries are almost occluded by the bacilli. We must assume, then, that although as a rule immediately before death all the conditions are present to enable the bacilli to multiply readily and to produce a large crop, this is not necessarily connected with the cause of death, being in fact in consequence of the animal being *in articulo mortis*; but that the immediate cause of death is the chemical alteration produced by the bacilli in the blood and tissues. For producing this effect it is not necessary to have more than a certain number of the bacilli. As soon as this number is reached death follows. The same may be said of other pathogenic organisms. Thus for instance in the case of tubercle-bacilli, after the introduction of these into the subcutaneous tissue of a guinea-pig, multiplication takes place, and after they reach a certain number, the nearest lymph-glands become swollen and inflamed and then caseous: but this stands in no relation to the number of the bacilli, for in some instances the microscopic examination reveals only very few bacilli, they are scattered in very small numbers over very wide areas. And the same is observed in the tuberculous deposits of the internal organs. In some of them the bacilli are exceedingly scarce, while in others neither more nor less advanced they are numerous. Here also we must assume that as soon as a certain, perhaps even small, number of the bacilli have been produced, the chemical effect produced is sufficient to be the cause of a certain pathological change. In glanders the nodules in the skin and lung reveal sometimes, even under the most careful examination after approved methods, the presence of but very few bacilli. In swine-plague, in

the lungs, which in severe cases are enormously affected, sometimes only very few of the pathogenic organisms can be discovered. It follows then that the pathological condition brought about by the organisms is not due to the direct action of their numbers; but is an indirect sequence, brought about by definite chemical alterations in the blood or tissues, as the case may be.

In this we may assume two theories as possible; (*a*) It is possible that these chemical effects are produced by the presence and growth of the organisms, as truly as in the alcoholic fermentation of sugar the alcohol produced is a result of the presence of the yeast; this change is only in so far a product of the organism as this, in its multiplication, assimilates some molecules of carbon and hydrogen, which it abstracts from the sugar, and in consequence of which the sugar yields alcohol; but it is not, as it were, a secretion of the organism, a special ferment. (*b*) But it is likewise possible that the organism elaborates a special ferment, which, after a certain amount has been produced, sets up the particular pathological changes. From these considerations it follows that the virus cannot be considered independent of the organism; we cannot assume that the two can have a separate existence; for, as we have just now shown, the most feasible assumption, and the one borne out by observation, is that owing to the multiplication of the organisms, certain chemical changes are produced in the blood and tissues, or that a special ferment is created, which sets up the anatomical changes characteristic of the particular disease.

CHAPTER XX.

VACCINATION AND IMMUNITY.

WE have in the foregoing chapter tried to show that owing to the presence in the normal blood and tissues of a living animal of some chemical substances varying in the different species and inimical to particular pathogenic organisms, the latter when introduced into the tissues of the particular species, cannot thrive, and that it is for this reason that the animal is not susceptible to the corresponding disease. Now, how do we explain the fact that a human being or an animal having been once the subject of a particular infectious disease, becomes thereby in some cases unsusceptible to a second attack? The oldest and perhaps the most favoured theory to explain this immunity is that which assumes that during the first attack the organisms growing in the body consume, or are instrumental in eliminating or destroying, some chemical compound necessary for the existence and multiplication of the organism. As soon as this substance has become consumed or destroyed the organisms cannot further multiply, and therefore the disease ceases; and further, that owing to the subsequent absence of this same chemical compound, a new infection by the same organisms is not possible, *i.e.* the individual is protected. Thus this theory puts the case on a

level with, say, the relation of the saccharomyces to the alcoholic fermentation; as long as a solution contains sugar, the saccharomyces is capable of multiplying, but as soon as all the sugar has disappeared as such, *i.e.* has become split up into alcohol and carbonic acid, the fermentation ceases, the solution being now exhausted as regards the saccharomyces; a new charge of saccharomyces put into the solution is not capable of multiplication. This theory, then, to explain the immunity, is generally spoken of as the *Exhaustion Theory*.

On careful analysis, it will be found that it is not capable of explaining all the facts of the case. As we mentioned in a former chapter, cattle inoculated with blood of a guinea-pig dead of anthrax become affected with anthrax, which, although not fatal, is nevertheless sometimes very severe. The animal recovers, and is now, for a time at least, protected against a second attack. But there is absolutely no ground for the assumption that if any infusion of the tissues of this animal were made, the bacillus anthracis sown in it would not thrive luxuriantly, seeing that bacillus anthracis grows on almost anything that contains a trace of proteids. Similarly when of the tissues of a guinea-pig, or mouse or rabbit, dead of anthrax, an infusion is made, and this is used as nourishing material for bacillus anthracis in artificial cultures, it is found that these latter thrive splendidly. The same fact I have observed in the case of swine-plague. There is then no reason whatever for assuming that, if after one attack of illness the blood and tissues become an unfavourable soil for a second invasion of the same organism, this should be due to the exhaustion of some necessary chemical compound.

There is another theory, commonly spoken of as the *Antidote Theory* (Klebs). According to this, the organisms

growing and multiplying in the body during the first attack produce, directly or indirectly, some substance which acts as a sort of poison against a second immigration of the same organism. I am inclined to think that this theory is in harmony with the facts. There is nothing known, from the observations before us, which would negative the possibility of the correctness of this theory; nay, I would almost say all our knowledge of the life of the micro-organisms points to the conclusion that the different species are associated with different kinds of chemical processes, and that as a result of the activity we find different chemical substances produced.

The different fermentations connected with the different species of fungi afford striking illustrations of this view. According to this theory, we can well understand that—just as in the case of an animal, say a pig, unsusceptible to anthrax—the unsusceptibility being due to the presence in the blood and tissues of a particular chemical substance inimical to the growth of the bacillus anthracis—so also in the case of a sheep or ox that has once passed through anthrax—there is now present in the blood and tissues a chemical substance inimical to the growth and multiplication of the bacillus anthracis whereby these animals become possessed of immunity against a second attack of anthrax.

Whether this chemical substance has been elaborated directly by the bacilli, or whether it is a result of the chemical processes induced in the body by the bacilli during the first illness, matters not at all; it is only necessary to assume that the blood and tissues of the living animal contain this chemical substance.

Some observers (Grawitz, &c.) are not satisfied with this theory, but assume that owing to the first attack the cells of the tissues so change their nature that they become capable

of resisting the immigration of a new generation of the same organism. There is absolutely nothing that I know of in favour of such a theory; it is impossible to imagine that the cells of the connective tissues, of the blood and of other organs, owing to a past attack of scarlatina, become possessed of new functions or of some new power, as, for instance, a greater power of oxidising or the like. Connective tissue-cells, blood-corpuscles, liver-cells, and other tissues are, so far as we know, possessed of precisely the same characters and functions after an attack of scarlatina as before.

On the whole then, we may, it seems, take it as probable, that owing to the presence in the normal blood and tissues *in a living animal of a chemical substance inimical to the* growth of a particular micro-organism, this animal is unsusceptible to the disease dependent on the growth and multiplication of this micro-organism; and further, that in those infectious maladies in which one attack gives immunity against a second attack of the same kind, one attack produces *a chemical substance in the blood and tissues* which acts inimically to a new immigration of the same organism; hence the animal becomes unsusceptible to a new attack, or is "protected." This is not the case with all infectious maladies, for, as is well known, in a good many instances a single attack does not protect against a second; and, as is also well known, a first attack may protect but only for a limited period, or for a period greatly differing in different individuals. All this would be explained by our theory in the same way as it is explained by the other theories; viz., when one attack does not protect, no inhibitory chemical substance has been produced; while in those diseases in which one attack does protect only for a limited period, the necessary inhibitory substance has only lasted for a limited period, and so on.

CHAPTER XXI.

ANTISEPTICS.

In former chapters we have on several occasions mentioned that a variety of substances and conditions are capable of exerting a detrimental influence on the life and growth of micro-organisms. Amongst these are—The presence of certain substances in the nutrient soil, the temperature, and some chemical products, such as those belonging to the aromatic series, phenol, indol, skatol, &c. The presence of certain substances in the nourishing material is, as we have seen, an essential condition, *cæteris paribus*, for the growth and multiplication of micro-organisms. Thus pathogenic organisms do not thrive in an acid medium, they cannot thrive if proteids or allied compounds and certain inorganic salts are absent: putrefactive and zymogenic organisms, on the other hand, or, at any rate, some of them, are capable of thriving well in acid media (*e.g.* the bacillus subtilis in acid hay-infusion, the micrococcus ureæ in acid urine). Further, many (not all) pathogenic organisms cannot thrive unless they are exposed to a certain degree of warmth; they thrive best at blood-heat, while putrefactive and many zymogenic organisms thrive well at ordinary temperatures, though of course their growth is more rapid

at higher temperatures, such as 30° to 38° C. Heat above 50° or 60° C. arrests the growth of and even kills many organisms, except the spores of bacilli, which, as we find on a former page, survive even when exposed to the temperature of boiling water for several minutes. The presence of carbolic acid, phenol, thymol, salicylic acid, perchloride of mercury, &c., restrain even when in great dilution the growth of micro-organisms.

In any inquiry into the influence of one substance or another on micro-organisms it is necessary to bear in mind that the influence of certain conditions on the micro-organisms may be a twofold one: (1) the condition may be unfavourable to the growth of the organism in question, and (2) the condition may be fatal to the life and existence of it. The second condition involves, *a fortiori*, the first; but the reverse is not the case. Owing to the failure to distinguish between these two propositions a great deal of confusion has arisen on the subject. One hears constantly this or that substance is an "antiseptic," meaning by this a substance unfavourable to the growth of micro-organisms, or a substance is a "germicide," implying by this that this substance kills the organisms; but when one comes to analyse the observations that are said to establish this reputation for a particular substance, one finds that the substances in question have really only a restraining effect on the growth of the organisms.

By sowing any micro-organism into a nourishing medium, to which has been added a certain substance (*e.g.* carbolic acid to the amount of 1 per cent.), and exposing this medium to the conditions of temperature, moisture, &c., otherwise favourable to the growth of the organism, if we find that after the lapse of a due period the growth is retarded or altogether inhibited, the conclusion is drawn that this substance (viz., the carbolic acid of 1 per cent.) is

a germicide. There is nothing more fallacious than this method of reasoning; a great many micro-organisms can be exposed to a 1 per cent. solution of carbolic acid for hours without in the least being affected, for on being then transferred to a suitable nourishing medium they grow and thrive well. Similarly by placing the spores of bacillus anthracis in a proteid medium containing perchloride of mercury of the strength of 1 in 300,000, it is found (as Koch has shown) that the spores are absolutely incapable of germinating. But if from this the conclusion is drawn, that perchloride of mercury of the strength of 1 in 300,000 is a germicide, I should most strongly dissent, for perchloride of mercury of 0·1 per cent. is not a germicide for all spores any more than vinegar; for on placing the spores of bacillus anthracis in a proteid medium, to which so much vinegar or any other acid has been added as makes it decidedly acid, it will be found that the spores do not germinate.

In order to pronounce a certain substance a germicide in the strict sense of the word, it is necessary to place the organisms in this substance for a definite time, then to remove them thence, and to place them in a suitable nourishing medium; if they then refuse to grow the conclusion is justified that the exposure has injured or destroyed the life of the organisms. In the case of pathogenic organisms a substance to be pronounced a germicide must be shown to have this power, that when the organism is exposed to the substance and then introduced into a suitable artificial medium it refuses to grow; and it must also be shown that when introduced into a suitable animal it is incapable of producing the disease which the same organism, unexposed to the substance in question, does produce.

I have made a good many observations on the influence

of antiseptics on micro-organisms, both putrefactive and pathogenic, and I have found that many assertions hitherto made on this subject, treated in the above light, are absolutely untrustworthy and erroneous.

Various species of putrefactive micrococci, bacterium termo, bacillus subtilis, various pathogenic micro-organisms, as bacillus anthracis, bacillus of swine-fever, absolutely refuse to grow in media to which is added phenyl-propionic acid, or phenyl-acetic acid, to an amount so small as 1 in 1,600; but if the same organisms are exposed to these substances in much stronger solutions, 1 in 800, 1 in 400, or even 1 in 200, and then transferred to a suitable nourishing material, it is found that they have completely retained their vitality, they multiply as if nothing had been done to them. I have exposed the spores of bacillus anthracis to the above acids of the strength of 1 in 200 for forty-eight hours and longer, and then inoculated guinea-pigs with them, and I found that the animals died of typical anthrax in exactly the same way as if they had been inoculated with pure spores of the bacillus anthracis.

Koch has published a large series of systematic and most valuable observations[1] made in testing the influence on spores of bacillus anthracis of a large number of antiseptics (thymol, arsenate of potassium, turpentine, clove-oil, iodine, hydrochloric acid, permanganate of potassium, eucalyptol, camphor, quinine, salicylic acid, benzoic acid, and many others), and amongst them he found perchloride of mercury to be the most powerful, since even a solution of 1 in 600,000 is capable of impeding, one of 1 in 300,000 of completely checking, the germinating power of the spores. To regard these substances, from these observations, in any way as germicides for the spores of bacillus anthracis would be

[1] *Mittheil. aus. d. k. Gesundheitsamte*, Berlin, 1881.

no more justifiable than to consider weak vinegar as such. Perchloride of mercury in a solution of 1 in 300,000 is no more capable of interfering with the life and functions of the spores of bacillus anthracis than water or salt solution, for the spores may be steeped in that solution for any length of time, and yet on being transferred to a suitable medium they grow and multiply splendidly, and when inoculated into rodents they produce fatal anthrax with absolute certainty. With my friend Dr. Blyth, Medical Officer of Health for the Marylebone district in London, I have tried the action of a number of substances in common use as antiseptics (*e.g.* Calvert's fluid, pure terebene, phenol 10 per cent., perchloride of mercury 0·1 per cent.) on the spores of bacillus anthracis, exposing these in comparatively large quantities of the above fluids (the two being well mixed) for several hours, and then inoculating guinea-pigs with them (spores and antiseptic). The animals died with symptoms of typical anthrax, the blood teeming with the bacillus anthracis.

These substances cannot therefore be considered germicides for the spores of bacillus anthracis, any more than water.

In all these inquiries, particularly in those upon pathogenic organisms capable of forming spores, the influence of the substances must be judged not merely by their action on the organisms, but also on the spores; for, in this very case of the bacillus anthracis, the bacilli taken from the blood of an animal dead of anthrax are killed after an exposure of say ten minutes to a solution of phenyl-propionic acid of the strength of 1 in 400, or even 1 in 800, whereas the spores of the bacilli (produced in artificial cultures) withstand completely exposure to this acid of any strength and for any length of time.

It is not my object to pass here in review all that has

been done in this interesting field of research, important because of its obviously great practical consequence. The work hitherto done has been enormous, but, I fear, of less utility than at first sight appears, for in most of it the point most prominent in the mind of the worker was to ascertain whether the particular antiseptic, mixed with the nourishing medium in a solution of definite strength, has or has not the power of inhibiting the growth of the micro-organisms. This point no doubt is of some interest, and perhaps of great interest, but whether a particular substance is an antiseptic in the proper sense of the word, *i.e.* whether on exposing the organisms to this substance in a solution of definite strength and for a definite period, the organisms become afterwards incapacitated from growing or multiplying; or still more, whether or not the substance is a germicide, *i.e.* is capable of altogether annihilating the life of the organisms; these are questions which require special attention, and represent a wide and rich field of inquiry; but, as far as I can see, have received only in very few instances due attention.

The disinfecting power of dry heat and steam on various pathogenic and non-pathogenic organisms has been studied by Koch and his coadjutors (*Mittheil. aus. d. k. Gesundheitsamte*, Berlin, 1881). Dr. Parsons has described in the supplement to the fourteenth annual *Report of the Medical Officer of the Local Government Board*, 1884, a series of observations made conjointly with myself on the influence of dry heat and steam on bacillus and spores of anthrax, bacillus of swine-fever and bacillus of tuberculosis. Pieces of flannel received the fluids containing the above organisms (separately), and were then exposed to heat. After this the organisms were squeezed out of the flannel previously steeped in salt solution, and this fluid was then injected into suitable animals and the effect watched, and compared with the result of the inoculation with the same materials not exposed to heat. The results of these experiments were (*l.c.* p. 225): "The spores of bacillus anthracis lost their pathogenic power after exposure for four hours to a temperature a little over the

boiling-point of water (212° to 216° F.), or for one hour to a temperature of 245° F. Non-spore bearing bacilli of anthrax (blood or culture) and of swine-fever, were rendered inert by exposure for an hour to a temperature of 212° to 218° F., and even five minutes exposure to this temperature sufficed to destroy the vitality of the former, and impair that of the latter." The tubercle bacilli were killed by exposure to dry heat for five minutes. Exposure to steam at 212° F. is destructive, even after five minutes, to all the pathogenic organisms tested, including the spores of bacillus anthracis.

The action of four solutions, phenylpropionic acid and phenylacetic acid, of phenylpropionate of soda and of sulpho-carbolate of soda, on bacillus anthracis, of blood and cultures, sporeless and spore-bearing, of phenylpropionic acid in conjunction with peptone, of acid reaction (sulphuric acid) on the life and growth of bacillus anthracis, the action of phenylpropionic and phenylacetic acid and sulpho-carbolate of soda on human and bovine tuberculous matter, of the action of chlorine and sulphurous acid gas on swine-fever virus is fully described in the *Report of the Medical Officer of the Local Government Board*, 1884, in a series of papers by myself, Mr. Laws, and Mr. A. Lingard.

The action of perchloride of mercury, although powerful, is not such as maintained by Koch (*Mittheil. aus. d. k. Gesundheitsamte*, Berlin, 1881). While Koch asserts that this substance in solutions of 1 in 100,000 has the power to kill sporeless bacillus anthracis, I find that exposure of the bacillus anthracis taken from the blood of an animal dead of anthrax to a solution of perchloride of mercury in water in the proportion of 1 in 20,000 or 1 in 25,000 for one hour does not impair the action of the bacilli. Solutions of 1 in 10,000 for thirty minutes' exposure kill the bacilli. But there exists a difference in resisting power according to the virulence of the bacilli themselves.

The bacilli of blood of an anthrax animal are capable of growing and of producing infective bacilli in broth peptone, and in nutrient gelatine to which perchloride of mercury has been previously added in the proportion of 1 in 25,000 or 1 in 30,000. The spores of bacillus anthracis are capable of germinating and producing good and active crops of bacilli in broth peptone, and in nutritive gelatine to which perchloride of mercury has been added in the proportion of 1 in 25,000 or 1 in 30,000. Koch mentions 1 in 300,000 as inhibiting the power of the germination of the spores of bacillus anthracis.

The spores of non-pathogenic bacilli and of some pathogenic bacilli (bacillus anthracis) resist the action of perchloride of mercury in solutions of 1 in 10,000 even after exposure for four days.

Non-pathogenic organisms have a markedly greater resisting power to phenylpropionic and phenylacetic acid and to perchloride of mercury than pathogenic.

Koch's comma bacilli of Asiatic cholera and Finkler's comma bacilli behave in this respect like non-pathogenic organisms. Sporeless non-pathogenic bacilli have a lesser resisting power than non-pathogenic micrococci.

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